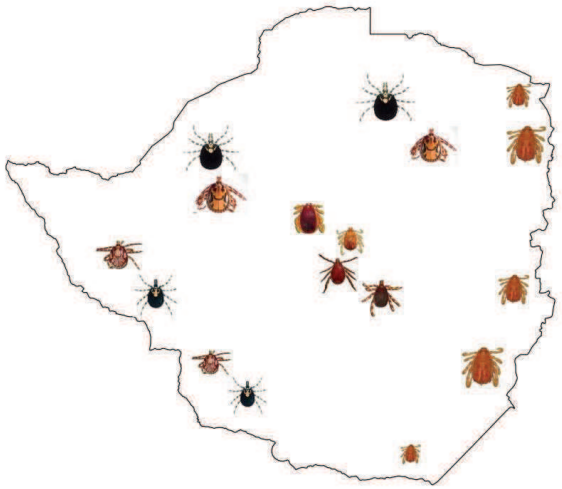
ISBN 978-9-4635704-7-3

Ixodid ticks parasitising cattle in Zimbabwe: Ecology and Management Marvelous Sungirai 2017

FACULTY OF BIOSCIENCE ENGINEERING



Ixodid ticks parasitising cattle in Zimbabwe: Ecology and Management

Marvelous Sungirai

GHENT UNIVERSITY

To the quest for knowledge

Promoters:	Prof. dr. ir. Patrick De Clercq Department of crop protection Faculty of Bioscience engineering Ghent University, Belgium
	Prof. dr. Maxime Madder Department of Veterinary Tropical Diseases Ondesterpoort Veterinary Institute University of Pretoria, South Africa
	Prof. dr. Doreen Zandile Moyo Department of Biological Sciences Midlands State University Gweru, Zimbabwe
Chair of the examination committee:	Prof. dr. ir. Wim Verbeke Department of Agricultural Economics Faculty of Bioscience Engineering Ghent University, Belgium
Members of the examination committee:	Prof. dr. ir. Dirk Berkvens, Unit Veterinary Biostatistics and Epidemiology Institute of Tropical Medicine Antwerp, Belgium
	Prof. dr. Edwin Claerebout, Faculty of Veterinary Medicine Ghent University, Belgium
	Prof. dr. Bertrand Losson, Department of Infectious and Parasitic Diseases Faculty of Veterinary Medicine University of Liège Liège, Belgium
	Prof. dr. ir. Thomas Van Leeuwen Dept of crop protection Faculty of Bioscience engineering Ghent University, Belgium
Dean:	Prof. dr. ir. Marc Van Meirvenne
Rector:	Prof. dr. ir. Rik Van de Walle

Dutch translation:

Harde teken (*Ixodidae*) als parasieten van rundvee in Zimbabwe: Ecologie en management

Please refer to this work as follows:

Sungirai, M. 2017. Ixodid ticks parasitising cattle in Zimbabwe: ecology and management. PhD thesis. Ghent University, Belgium

Front cover: Zimbabwe map showing some of the tick species collected.

Back cover: Cattle at a communal dip tank.

ISBN-number: 978-94-6357-047-3

This research was conducted in Zimbabwe and at the laboratory of the Institute of Tropical Medicine, Campus Mortelmans, Antwerp, Belgium.

Acknowledgements

A lot of people contributed to this work and Iam forever indebted to them.

Professor M. Madder, through whom the ideas for this work were conceived way back in 2012 when I had just finished my Masters. He urged and encouraged me to aim higher.

Professor D.Z. Moyo, for the professional and at times emotional support as the going got tough.

Professor P. De Clercq, for sharpening my writing skills, thorough, expeditious comments and the constructive criticism.

Professor C. Maritz-Olivier, Thank you for giving me access to your lab, the training I received and the discussions we had contributed immensely to the success of this work.

The whole network of the Department of Veterinary Services in Zimbabwe, the Deputy Director Research and Diagnostics Dr P.V. Makaya, the provincial and district veterinary officers and animal health inspectors. You all enabled me in one way or other to collect the tick samples at the communal dipping tanks distributed throughout the country.

The laboratory staff at the Institute of Tropical Medicine (ITM) especially Jacobus de Witte. Thank you lots for providing a helping hand whenever I needed one.

The unit of Epidemiology and Biostatistics and the formerly unit of Veterinary Entomology, thank you for the companionship.

Nadia Ehlinger and Danielle De Boise. A big thank you for all the help with all the administrative procedures. I appreciate.

The student service at the ITM, thank you for all the assistance and making my stay in Belgium comfortable.

The Midlands State University leadership. I wish to extend my profound gratitude to you for allowing me to have this opportunity to improve my academic career.

The Department of Development Co-operation (DGD) of the Belgian government. Thank you for the financial support which made this work a reality.

Rudo, Abigail, Angel, Adonai and Aisha my shining beacons, this is for you!!! My father, late mother (who passed away in the midst of this journey) and my siblings, you are a source of inspiration, you make me aim higher and I will always take you with me. My colleagues in the Department of Livestock and Wildlife Management at Midlands State University, thank you for always standing for me. Iam forever grateful.

To everyone I met in Belgium and made my stay comfortable. Tapiwa Ganyani, many thanks for proof reading the manuscript. You are a true friend!!!Lots of success with your PhD.

To God be the glory!!!

Marvelous Sungirai, November 2017

Table of Contents

Abstract iv
List of Abbreviations vi
Chapter 1: Introduction, Objectives and Thesis Outline 1
1.1. General Introduction 1
1.2. Background to the study 4
1.3. Objectives of the study 5
1.3.1. Hypotheses 6
1.4. Thesis outline 6
Chapter 2: Distribution of ixodid ticks, population structure and tick control strategies: A literature review 8
2.1 Ixodid ticks of Southern Africa 8
2.1.1. Identification of ixodid ticks 14
2.2. Distribution of ixodid ticks in southern Africa 16
2.3. Predictive modelling of tick distributions 18
2.4. Population genetics of ixodid ticks 22
2.5. Participatory epidemiology 23
2.6. Acaricides in the control of cattle ticks 25
2.6.1. Mechanism of action of common acaricides used to control cattle ticks 26
2.6.2. Application of acaricides on cattle 30
2.6.3. Factors influencing frequency of acaricide application 32
2.6.4. Acaricide resistance in ixodid ticks 33
2.6.5. Diagnosis of acaricide resistance 37
2.7. Alternative tick control methods 42
Chapter 3: Shifts in the distribution of ixodid ticks parasitizing cattle in Zimbabwe 44
3.1. Introduction 45
3.2. Materials and Methods 46
3.2.1. Study area and sampling 46
3.2.2. Data analysis 49
3.3. Results 49
3.3.1. Dip tank prevalence of ixodid tick species 49
3.3.2. Ecological distribution of ixodid tick species 50

3.3.3. Prevalence of ticks on cattle 52
3.4. Discussion 55
Chapter 4: Modelling the distribution of *Rhipicephalus microplus* and *Rhipicephalus decoloratus* in Zimbabwe 61
4.1. Introduction 61
4.2. Methodology 63
4.2.1. Study area 63
4.2.2. Environmental variables 63
4.2.3. Model building 64
4.2.4. Model Evaluation 64
4.2.5. Environmental requirements for the two tick species 65
4.2.6. Software used 65
4.3. Results 65
4.3.1. Univariate analysis 65
4.3.2. Multivariate Analysis 66
4.3.3. Habitat suitability maps 68
4.3.4. Environmental requirements for the two tick species 70
4.4. Discussion 73
Chapter 5: Population structure and genetic diversity of *Rhipicephalus microplus* in Zimbabwe…… 77
5.1. Introduction 77
5.2. Materials and Methods 78
5.2.1. Biological materials and DNA extraction 78
5.2.2. Microsatellite Selection and data analysis 79
5.3. Results 81
5.3.1. Microsatellite selection and data analysis 81
5.4. Genetic differentiation and population structure 82
5.5. Discussion 86
Chapter 6: Communal farmers' perceptions of tick-borne diseases affecting cattle and investigation of tick control methods practiced in Zimbabwe 88
6.1. Introduction 89
6.2. Materials and Methods 91
6.2.1. Study area 91
6.2.2. Data collection 92

6.2.3. Sampling 94
6.2.4. Data analysis 95
6.3. Results 95
6.3.1. Demographics and summary of major responses 95
6.3.2. Perception of ticks, tick-borne and related diseases by communal farmers 98
6.3.3. Factors influencing knowledge of TBDs and use of other tick control methods 100
6.4. Discussion 102
Chapter 7: Genotyping acaricide resistance profiles of *Rhipicephalus microplus* tick populations from communal areas of Zimbabwe 107
7.1. Introduction 108
7.2. Materials and Methods 110
7.2.1. Biological material and DNA extraction 110
7.2.2. PCR conditions for the octopamine/tyramine receptor gene, carboxylesterase genes and voltage-gated sodium channel genes 111
7.2.3. PCR cloning and sequencing 111
7.2.4. Data Analysis 112
7.3. Results 113
7.3.1. Single Nucleotide Polymorphisms (SNPs) in the octopamine / tyramine receptor gene 113
7.3.2. Single Nucleotide Polymorphism in the carboxylesterase gene 115
7.3.3. Genotype Frequencies and Hardy Weinberg Equilibrium 117
7.4. Discussion 120
Chapter 8: General Discussion, Conclusion and Recommendations 123
Summary 131
Samenvatting 135
References 139
Curriculum Vitae 169

Abstract

A nationwide survey was conducted in Zimbabwe between September 2013 and May 2015 to determine the ecological distribution of ixodid tick species parasitising cattle. Thirteen tick species were collected and identified with notable changes in the distribution of *Amblyomma hebraeum*, *Amblyomma variegatum* and *Rhipicephalus microplus* being observed. Habitat suitability models were then built to compare the spatial limits of the invasive *R. microplus* species and its autochthonous competitor *R. decoloratus*. The results indicated that *R. microplus* had reached its spatial limit and in suitable areas it would be expected to displace *R. decoloratus*. Partial displacement was observed in the study as the two tick species co-existed in 50% of the study areas, suggesting that non-climatic factors are playing a role in the spatial occurrence of the two tick species. Population genetics was used to assess variation and differentiation of *R. microplus* tick populations. A weak population structure was observed (F_{ST}=0-0.08) with most of the variation (97%) being found within populations. Additionally high levels of heterozygosity (0.76-0.8) in the individual populations suggested increased gene flow and recent population expansion which is facilitated by continuous cattle movement. Farmers' views and management of ticks and TBDs were assessed through a questionnaire survey with 313 participants. There was a high level (67.7%) of awareness of ticks and tick-borne diseases (TBDs) by the farmers, with high participation in tick control programmes (70.3%), use of alternative tick control methods (59.4%) and high frequency of use (67.4%) of one acaricide type, amitraz. It was concluded that these factors would predispose tick populations to acaricide resistance. To assess whether these populations were undergoing selection pressure for resistance, molecular markers associated with resistance to amitraz, pyrethroids and organophosphate were used to identify Single Nucloetide Polymorphism (SNPs) and genotype the individuals. SNPs associated with resistance to amitraz, pyrethroids and organophosphate were identified in the octopamine/tyramine receptor and carboxylesterase markers respectively. The frequency of the mutant allele associated with resistance to amidines (amitraz) was comparably higher (55%) to the one found at the carboxylesterase marker (5.4%). These results suggested that the *R. microplus* population was undergoing selection pressure for amitraz resistance although a large proportion (78%) of tick samples had heterozygous genotypes indicating balancing

selection. Mutations previously reported to be associated with pyrethroid resistance in the voltage-gated sodium channel gene were not found in Zimbabwean tick samples. This suggested a different mechanism of resistance towards pyrethroids or low levels of resistance in the tick populations. It is concluded that periodic surveillance for tick dispersal will continue to give us new information which will be important in the implementation of area-specific tick control programmes depending on tick diversity and abundance. With *R. microplus* having reached its spatial limits, the high levels of gene flow observed will suggest that sporadic occurrences will be found in unusual areas as noted in this study. These findings have implications on the epidemiology of TBDs, their management and control in communal settings in general and Zimbabwe in particular.

List of Abbreviations

Ache	Acetylcholine esterase
AIC	Akaike Information Criterion
AIT	Adult Immersion Test
AMOVA	Analysis of Molecular Variance
AUC	Area Under the Curve
AVHRRI	Advanced Very High Resolution Radiometer
BHC	Benzene Hexane Chloride
Bt	Bacillus thuringeiensis
Cae	Carboxylesterase
CCHF	Crimean Congo Haemorrhagic Fever
COX	Cytochrome Oxidase
DDT	Dichlorodiphenyltrichloroethane
DEM	Diethylmaleate
dLST	daytime Land Surface Temperature
DNA	Deoxyribonucleic Acid
DVS	Department of Veterinary Services
ECF	East Coast Fever
GABA	Gamma Amino Butyric Acid
GDP	Gross Domestic Product
GIS	Geographic Information System
GST	Glutathione S-Transferases
HWE	Hardy Weinberg Equilibrium
ITS	Internal Transcribed Spacer
JH	Juvenile Hormone
kdr	knock down resistance
LD	Linkage Disequilibrium
LDC	Livestock Development Committee
LIT	Larval Immersion Test
LPT	Larval Packet Test
LSD	Lumpy Skin Disease
LTT	Larval Tarsal Test
MCMC	Markov Chain Monte Carlo
MFOs	Multi -Function Oxidases
MODIS	Moderate Resolution Imaging Spectroradiometer
NDVI	Normalised Difference Vegetation Index
NOAA	National Oceanic and Atmospheric Administration
Oct/Tyr	Octopamine/Tyramine
OP	Organophosphate
PBO	Piperonyl Butoxide
PCoA	Principal Co-ordinate Analysis
PCR	Polymerase Chain Reaction
PE	Participatory Epidemiology
RFLP	Restricted Fragment Length Polymorphism
ROC	Receiver Operating Characteristic
RPCF	Resource Poor Communal Farmer
SNPs	Single Nucleotide Polymorphisms
SSR	Simple Sequence Repeats
TBDs	Tick borne diseases
TPP	Triphenyl Phosphate
VGS	Voltage-gated Sodium Channel

Chapter 1: Introduction, Objectives and Thesis Outline

1.1. General Introduction

Ticks are obligate blood feeding ectoparasites of vertebrate mammals, birds and reptiles (Wall and Shearer, 2001). They belong to the phylum of Arthropods in the class Arachnida, sub-class Acari, order Parasitiformes, sub-order Ixodida. The latter is sub-divided into 3 families: Ixodidae (hard ticks), Argasidae (soft ticks) and the Nuttalliellidae which has only one tick species (Walker et al., 2000). There are about 900 species of ticks which have been described globally (Guglielmone et al., 2010) with six of these genera being hard ticks of veterinary importance, namely *Dermacentor, Haemaphysalis, Hyalomma, Rhipicephalus* (including *Boophilus*)*, Ixodes* and *Amblyomma* (Walker et al., 2003).

Ixodid ticks affect human and animal health worldwide (Jongejan and Uilenberg, 2004) and are responsible for losses in the livestock industry running into millions of USD$ annually, particularly in (sub) tropical areas (Mukhebi et al., 1999), although these figures are often underestimated (Jongejan and Uilenberg, 2004). These losses are due to direct effects of ticks as blood sucking parasites which leads to reduction in live weight, anemia and poor quality hides and indirect effects as both biological and mechanical vectors of diseases that affect animal performance and can lead to cattle mortalities (Rajput et al., 2006). Indirect losses are also incurred in the control and treatment of ticks and tick-borne diseases (TBDs) (de Castro, 1997). For example in Zimbabwe, the annual costs of acaricides to control cattle ticks have been estimated at US$9.63 per animal (Pegram et al., 1996).

Agriculture is the mainstay of economies of most developing countries with livestock and particularly cattle production contributing significantly to the overall contribution of agriculture to the countries' Gross Domestic Product (GDP) (Thornton, 2010). Poor nutrition and health have been cited as the major factors impeding the development of the livestock industry in these emerging economies (Lamy et al., 2012). In particular in as far as health issues are concerned, TBDs have been identified as the most important animal health management problem in Africa (Young et al., 1988). Tick borne diseases

account for the majority of cattle mortalities and resource poor farmers who own the majority of cattle are most affected with 80% of their cattle at risk of TBDs (Rushton et al., 2002). Hence it is important to come up with strategies for the effective control of these diseases.

Globally, changes in the spatial distribution of ixodid ticks have occurred, and have been principally caused by climate change, habitat modifications and human activities (Lèger et al., 2013). Both survival and reproduction of ticks in an area are dependent on biotic and abiotic conditions. The presence of suitable hosts is essential for feeding and appropriate environmental conditions of temperature and humidity ensure survival during the free-living phase of the ticks' life cycle. While the movement of hosts plays a crucial role in tick dispersal to new environments (Barré and Uilenberg, 2010), it is the ability of the tick species to adapt to climatic conditions of the area and the continued availability of hosts that ensure its establishment (Cumming, 1999). Therefore it is imperative to understand the environmental and climate niche of the tick species in light of their present known spatial distribution (Estrada-Peña, 2008). This will help in predicting disease occurrence and emergence (Lèger et al., 2013) as well as in developing effective area specific surveillance and control programmes (De Clercq et al., 2015).

It is important to consider tick population dynamics and dispersal when reflecting on the effectiveness of tick control for reducing pathogen transmission (Araya-Anchetta et al., 2015). Such a consideration may provide valuable insights into the evolutionary forces which have been driving gene flow (McCoy, 2008), help describe the distribution of species and their spatial limits (Chevillon et al., 2013) and also help explain host adaptation which might have a bearing on disease specificity (Gandon and Michalakis, 2002) as well as on resistance to acaricides (Roush and Daly, 1990). Generally it is hypothesised that parasites will occur in small homogenous populations with little gene flow between populations but such hypotheses could be confounded by adaptation to habitat heterogeneity, host movement, large population sizes and expansion of host home ranges (Mixson et al., 2006). However, because of their small size, location, behaviour and biology the direct observation of the population biology of ticks is almost impossible and indirect methods have proved to be useful (De Meeùs et al., 2007). Indirect methods use neutral polymorphic molecular markers to measure variation within

and between defined populations where upon the distribution of genetic variation should reflect ecologically relevant population parameters (Araya-Anchetta et al., 2015).

Cattle owners play a very important role in the control of livestock diseases (De Garine-Wichatitsky et al., 2013) and their involvement in identifying problems and designing solutions to those problems is beneficial in the success of any animal health intervention (Catley et al., 2012). As such, knowledge, attitudes and perceptions of diseases by farmers is very important in coming up with effective community animal health programmes. It has been observed that resource poor communal farmers provide more than 80% of information used for surveillance in most countries (Grace et al., 2008). Since the late 1990s the field of participatory epidemiology has evolved, which is defined by Catley et al. (2012) as, "the systematic use of participatory approaches and methods to improve understanding of diseases and options for animal disease control". The stakeholders are empowered to identify and solve their own problems (Mariner et al., 2011) and this has been viewed as a cheap form of data collection (Thrusfield, 2005). Data collection in developing countries is often hampered by poor laboratory diagnostic support, insufficient manpower and sometimes difficult terrain (Thrusfield, 2005). Hence, to achieve better control of ticks and TBDs affecting resource poor cattle farmers it is important to understand their knowledge, attitudes and perceptions.

The control of ticks has relied upon the use of chemicals known as acaricides which was heralded by the use of the chemical arsenic at the end of the 19th century (George, 2000). Since then, several acaricide products for the control of ticks have been introduced (George et al., 2004), however development of resistance has reduced their efficacy (Rosario-Cruz et al., 2009). Detection of resistance is a major component of any effective ectoparasite control programme (Faza et al., 2013) as it guides the selection of an appropriate acaricide which will be effective in resistance management (Guerrero and Pruett, 2003). The advent of Polymerase Chain Reaction (PCR) based molecular tools has enabled the rapid detection of the resistance status of tick samples. These tools will enable the genotyping of acaricide resistance status, going beyond the bioassay methods which only allow the phenotypic determination of acaricide resistance. In addition, the latter methods are laborious and time consuming and require the presence of live tick specimens (Higa et al., 2015).

1.2. Background to the study

Zimbabwe has witnessed land use and ownership changes facilitated by the fast track Land Reform Programme which began at the end of the year 1999 (Scoones et al, 2010). For the livestock industry, this was accompanied by unsanctioned livestock movements (Mavedzenge et al, 2006) which are an important medium for tick dispersal, particularly the one host ticks (Barré and Uilenberg, 2010). As a result this would create a situation whereby vectors and the pathogens they transmit will occur in areas that they were not known to occur. Studies have been carried out to determine tick distribution in the country (Katsande et al., 1996; Peter et al., 1998a), however there is need for updated information which highlights the current situation in as far as the zoogeography of ticks is concerned. Over the years, due to the worsening economic situation, research on the ecology of ticks and the epidemiology of the diseases they transmit is lacking and decisions are informed by past records which may be outdated. Knowledge on tick distribution is vital especially for animal health management authorities in planning vector and disease control programs. The Zimbabwe government spends millions of United States Dollars annually on tick control programs, which have been premised on the traditional and conventional weekly interval dipping during the wet season and forty-nightly intervals during the dry season (Norval et al, 1992a). However, without knowledge on recent tick distributions and in-depth studies on seasonal tick burden taking into account environmental variables such as changes in climate as well as livestock movements, it is difficult to decide whether or not to continue with traditional control practices.

The most important cattle tick recognised globally is *Rhipicephalus microplus* which is commonly known as the cattle tick (Angus, 1996). This is a one-host tick of Asiatic origin which through livestock migrations is now established in many tropical and sub-tropical countries (Barré and Uilenberg, 2010). In Zimbabwe it has been known to inhabit the humid eastern highlands where there is a favourable condition for its proliferation after introduction from Mozambique in the 1970s (Mason & Norval, 1980). The tick is known to be highly invasive and will displace locally known tick species of the *Boophilus* sub-genus (Madder et al., 2011). In Zimbabwe there is little information on the adaptable character of *R. microplus* to the dry areas which might consequently influence the epidemiology of bovine babesiosis. It had been reported in the 70s that *R. microplus*

was displacing *R. decoloratus* but this was only as far as the areas in which the tick ecologically thrived (Mason & Norval, 1980). There were indications that successive droughts which occurred in Zimbabwe between 1980 and 1984 had resulted in the disappearance of *R. microplus* in the areas in which it had occurred previously, however subsequent reports indicated that *R. microplus* was spreading from the east to the central parts of the country (Katsande et al., 1996). Smeenk et al. (2000) suggested that *R. microplus* seems to periodically spread into the inner parts of the country and highlighted that there is need to investigate whether it had established in those areas. The acaricidal control of ticks has been hampered by the emergence of resistant strains which has been reported in several countries especially for the cattle tick *Rhipicephalus microplus* (Abbas et al., 2014; Guerrero et al., 2012). Due to the short generation time in one host ticks and the fact that these tick stages from larvae to adult are spent on the host, selection pressure will be more important in these tick species as compared to multi-hosts ticks (Mekonnen et al., 2002). In Zimbabwe, there is need to gather information on the different chemicals being used by communal farmers as well as investigate how they are managing ticks and TBDs in their respective areas especially in light of economic structural adjustment programmes which have led to reduction in veterinary support services (Peter et al, 2005) . It is also important to investigate the levels of acaricide resistance to the different chemicals being used by farmers whose diagnosis has been enhanced by the development of molecular tools (Guerrero et al., 2001; Hernandez et al., 2002; Baron et al., 2015).

1.3. Objectives of the study

The objectives of this study were to determine the spatial distribution of ixodid ticks parasitising cattle in Zimbabwe and compare with previously published studies to conclude whether they have been any changes. The study also sought to investigate the habitat suitability of the invasive *Rhipicephalus microplus* and the local *Rhipicephalus decoloratus* tick so as to determine their spatial limits and regions of overlap. Furthermore, the study sought to use population genetic tools to investigate variability and differentiation of *R. microplus* tick populations in Zimbabwe. Lastly, the study sought to investigate the management of ticks and TBDs by communal farmers and whether there was selection pressure for resistance towards the acaricide chemicals being used.

The main research questions are:

1. Have there been shifts in the spatial distribution of ixodid tick species parasitising cattle in Zimbabwe?
2. What are the spatial limits and regions of overlap for *R. microplus* and *R. decoloratus* in Zimbabwe?
3. Are *R. microplus* populations genetically differentiated across geographic scales in Zimbabwe?
4. How do resource poor farmers manage ticks and TBDs affecting their cattle?
5. Are *R. microplus* populations undergoing selection pressure for acaricide resistance?
6.

1.3.1. Hypotheses

The hypotheses driving this study are that geospatial and temporal changes have led to a shift in the distribution of ixodid ticks parasitising cattle in communal land areas of Zimbabwe. Invasive tick species may subsequently occupy regions where local tick species were established and their populations will be genetically differentiated as an adaptation strategy to new environments. The farmers' views on ticks and TBDs as well as their management has evolved, with tick populations going through selection pressure for acaricide resistance.

1.4. Thesis outline

Chapter 2 is a comprehensive literature review which provides current information on the known distribution of ixodid ticks and their importance to the cattle industry in southern Africa. The chapter further presents progress made in studying the ecology of ixodid ticks in relation to the factors influencing their spatial distribution. The chapter also discusses the implementation of species distribution modelling to determine suitable habitats for tick species as well as how population genetics plays a crucial role in understanding vector biology, host-parasite relationships, invasion of parasites to new areas and evolutionary adaptation in those areas. Furthermore, the chapter also discusses the role of participatory epidemiology in the control of ticks and TBDs as well as the management and control strategies of ticks and TBDs in developing countries.

Chapter 3 describes a nationwide survey of tick distribution highlighting the major changes that have occurred since the last published survey. **Chapter 4** looks at the habitat suitability of the invasive tick *R. microplus* together with its competitor species *R. decoloratus* and compares the environmental requirements of the two species in Zimbabwe. **Chapter 5** investigates genetic diversity and differentiation in Zimbabwe *R. microplus* tick populations. **Chapter 6** looks at the management of ticks and tick borne diseases by resource poor communal farmers. **Chapter 7** investigates whether *R. microplus* tick populations are undergoing selection pressure using molecular markers that have been associated with resistance. The thesis ends with a general discussion in **Chapter 8** and a reflection on possible research areas which may improve the management of ticks and TBDs in developing countries especially in resource poor farming communities.

Chapter 2: Distribution of ixodid ticks, population structure and tick control strategies: A literature review

2.1 Ixodid ticks of Southern Africa

The development of the livestock industry in most tropical countries suffers a setback due to diseases of which TBDs rank highly (Pegram et al., 1993). The major TBDs affecting livestock in tropical countries are heartwater (cowdriosis), gall sickness (anaplasmosis), red water (babesiosis) and east coast fever (theileriosis) (Minjauw and Mcleod, 2003). These diseases are responsible for huge economic losses in the livestock industry which have been conservatively put at millions of USD annually (Rushton et al., 2002). Southern Africa is home to millions of livestock and wildlife that together serve as principal hosts to a number of ixodid tick species of which those in the genera *Rhipicephalus*, *Amblyomma*, *Hyalomma, Ixodes* and *Haemaphysalis* are of veterinary importance (Walker, 1991). These tick species serve as both biological and mechanical vectors of pathogens which cause the above-mentioned diseases.

Studies on the geographic distribution of ixodid ticks carried out in Southern Africa over the past years have helped in describing the spatial occurrence and habitat suitability of these obligate parasites (Berkvens et al., 1998; Estrada-Peña et al., 2008; Horak et al., 2009; Norval, 1983; Peter et al., 1998a; Speybroeck et al., 2002). Ixodid ticks can be described by their life cycle as: one-, two- or three-host ticks (Figure 2-1 and Figure 2-2) depending on whether or not they drop off after engorgement during the developmental stages from larvae to adults (Walker et al., 2007). One host ticks will only quest for a host in the larval stage while 2- and 3-host ticks will quest in larval and adult stage or in the larval, nymphal and adult stage, respectively. It has been observed that the distribution of one-host ticks like *Rhipicephalus microplus* is significantly influenced by the movement of host animals from one area to another (Barré and Uilenberg, 2010).

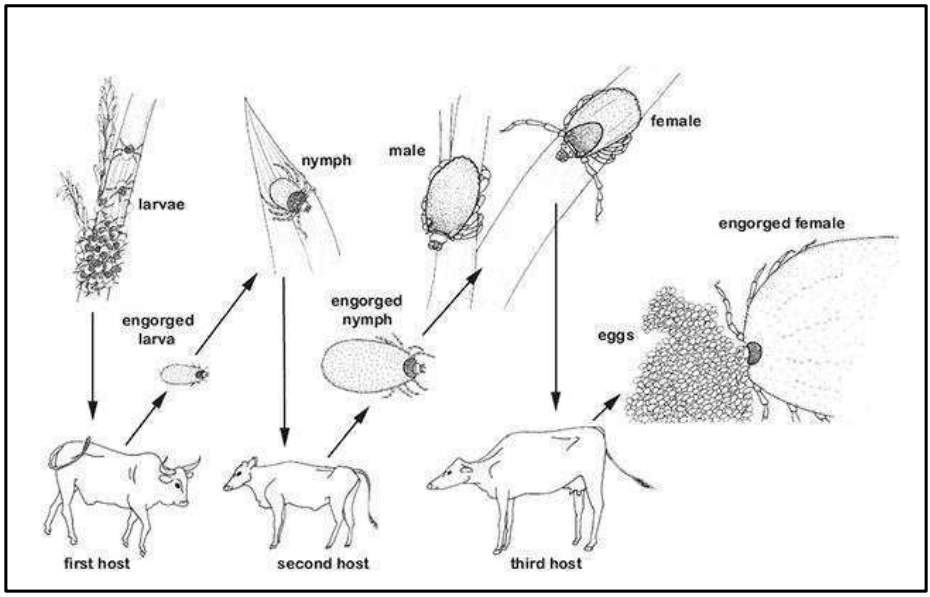


Figure 2-1: Life cycle of a three- host tick (e.g. *R. appendiculatus*, source Walker et al., 2003)

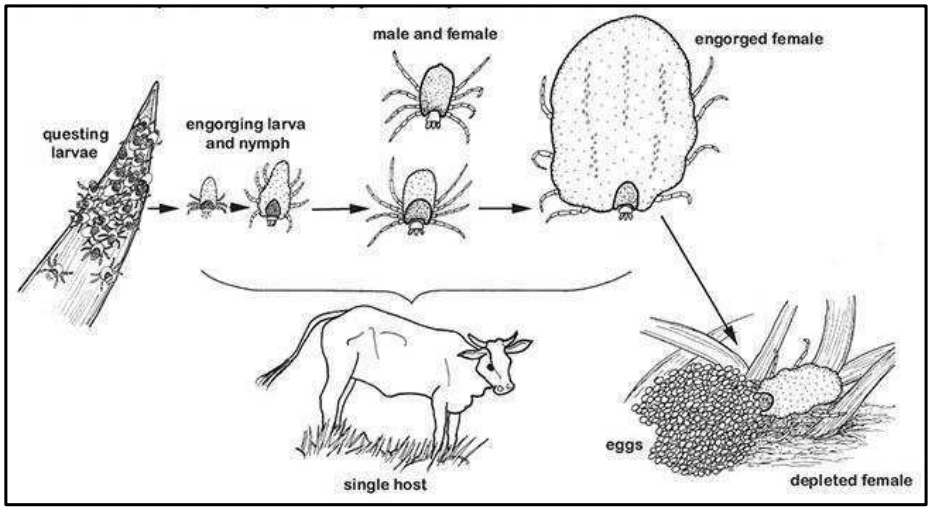


Figure 2-2: Life cycle of a one -host tick (e.g. *R. microplus*, source Walker et al., 2003)

The genus *Rhipicephalus* (brown ticks) is the fourth largest of the tick genera comprising 74 tick species widely distributed throughout the world (Olwoch et al., 2007) and it is the largest genus in Southern Africa with 28 described species in the region (Walker, 1991). The most important tick species in this genus are *Rhipicephalus appendiculatus*,

Rhipicephalus zambeziensis, *Rhipicephalus sanguineus*, *Rhipicephalus simus* and *Rhipicephalus evertsi evertsi*. These are largely 2- or 3-host tick species (with exception of the subgenus *Boophilus*) which play pivotal roles in the transmission of the diseases theileriosis (*R. appendiculatus*, *R. zambeziensis*, *R. dutonni*), biliary fever (*Babesia canis* in dogs transmitted by *R. sanguineus*) and anaplasmosis (*R. evertsi evertsi* and *R. simus*). In the genus *Rhipicephalus* is a group of one-host ticks popularly known as the blue ticks. These species previously used to be grouped in a separate genus known as *Boophilus* but molecular taxonomy showed them being monophyletic with the genus *Rhipicephalus* (Murrell et al., 2000) and this led them to be placed in the genus *Rhipicephalus* with the name *Boophilus* being retained as a sub-generic epithet (Horak et al., 2003). In Africa, there are four species belonging to this group, namely *R. microplus*, *R. decoloratus*, *R. annulatus* and *R. geigyi*. *Rhipicephalus decoloratus* and *R. microplus* have a greater distribution in Africa being present in large parts of sub-Saharan Africa whilst *R. geigyi* and *R. annulatus* are restricted to the western parts of the continent (Walker et al., 2003).

Evolutionary, *Rhipicephalus decoloratus* and *R. geigyi* are autochthonous to the African continent whilst *R. microplus* and *R. annulatus* are introduced species from Asia with cattle movements (importations) after the rinderpest epidemic in the late nineteenth century playing a significant role in their spread to the African continent (Jongejan and Uilenberg, 2004). The biology of these tick species makes them one of the important veterinary pests of the world. As one-host ticks most of the life-cycle (from larva to adult, roughly 3 weeks) is spent on the animal hence their dispersal is much enhanced by animal movements (Barré and Uilenberg, 2010) and they have an increased frequency of exposure to acaricides making them susceptible to acaricide resistance, which is less so with the 2- or 3-host ticks (Mekonnen et al., 2002). In addition, they have a high reproductive capacity characterised by shorter life-cycles leading to 3 or 4 generations per year (Oliver Jr, 1989), in sharp contrast to the 2- or 3-host ticks which can have one generation in more than 12 months (Walker et al., 2000).

The boophilids, *R. microplus* and *R. decoloratus* are the only blue ticks found in Southern Africa and they influence the epidemiology of bovine babesiosis and anaplasmosis (Estrada-Peña et al., 2006a). *Rhipicephalus microplus* transmits *Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale* which cause babesiosis and anaplasmosis, respectively, whilst *R. decoloratus* transmits *Babesia bigemina* which causes the less pathogenic form of babesiosis (Mason and Norval, 1980) and *A. marginale* which causes anaplasmosis. Endemic stability to babesiosis is observed especially in the indigenous and tropically adaptable *Bos indicus* cattle breeds but the introduction of *R. microplus* to susceptible cattle (without previous immune protection), particularly the exotic *Bos taurus* cattle, will lead to epidemics resulting in substantial cattle mortalities (De Vos and Potgieter, 1994). The impacts of *R. microplus* intrusion in cattle areas have been reported in several African countries (Madder et al., 2012; Nyangiwe et al., 2013a; Tønnesen et al., 2004) and elsewhere in the world, particularly in Latin America, and the highly invasive character of this species, its rapid development of acaricide resistance and ability to adapt to other host species has attracted the attention of acarologists worldwide (Chevillon et al., 2013; Lèger et al., 2013).

One of the most important tick-borne diseases in Southern Africa is theileriosis which depending on the epidemiology could be referred to as January disease (Zimbabwean theileriosis), Corridor disease (buffalo associated theileriosis) and East Coast Fever (ECF) (Norval et al., 1992b). Theileriosis is caused by the protozoan parasite *Theileria parva* which is originally a parasite of African Buffalo (*Syncerus caffer*) where infected buffaloes are asymptomatic and remain long term healthy carriers (Uilenberg, 1999). January disease and ECF are cattle derived, with transmission occurring from cattle to cattle. However, they differ in their virulence, this is largely due to a phenomenon called diapause. Diapause occurs in unfed adult ticks which do not attach or quest for hosts until the emergence of the appropriate environmental conditions for their survival (Randolph, 2010). This diapause behaviour is only observed in Southern Africa *R. appendiculatus* ticks resulting in one generation per year due to a prolonged cold dry season (Madder et al., 2002).

In East Africa as a result of the absence of the long cold dry season, diapause does not occur resulting in more than one generation of *R. appendiculatus* ticks in a year (Ochanda et al., 1998). The virulent strain of *T. parva* can only be acquired from cattle

by larvae to nymphs and transmitted to adults only when there is an overlap of generations. Therefore the existence of more than one life stage at any given time will maintain the virulent strain in East Africa. Although transmission in Southern Africa will occur from nymphs to adults once a year and during the diapause phase, the ticks would lose their infectivity (Norval et al., 1991a) and thus become less virulent as compared to those observed in the Eastern or equatorial regions. ECF has been eradicated in Southern Africa, while January disease still persists in Zimbabwe. Due to the wide distribution of the African Buffalo in Southern Africa, corridor disease still occurs as a result of frequent contacts of buffaloes with cattle along the livestock-wildlife interface. Theileriosis is characterised by endemically unstable situations which results in mortalities in young animals which are not immune protected.

Cattle, small ruminants and wildlife species in Africa are prone to infections with cowdriosis. Cowdriosis (heartwater) is caused by the gram negative bacteria *Erlichia* (formerly *Cowdria*) *ruminantium* which is characterised by high fever, diarrhoea and nervous signs with a typical necropsy of accumulation of fluid in the hydro-pericardium (Deem, 1998). The vectors for this disease are the tropical bont tick (*Amblyomma variegatum*) and the South African bont tick *Amblyomma hebraeum*. Whereas the latter is widely distributed in Southern Africa (Petney et al., 1987), the former has a more widespread African distribution and was introduced to the Caribbean Islands in the 19th century (Lèger et al., 2013). The ticks in the genus *Amblyomma* are 3-host ticks which cause debilitating losses not only as vectors but also as blood sucking parasites causing extensive damage to the skin and udder of cattle. This may also lead to secondary infections with mastitis resulting in significant reductions in milk yield of lactating cows. *Amblyomma variegatum* has been associated with the cattle disease dermatophilosis (Norval, 1983) which affects the skin and may also cause mortalities. Tick bites due to *A. variegatum* may open up wounds which act as portals of entry for the bacterium *Dermatophilus congolensis*. Preliminary investigations have revealed that *Amblyomma hebraeum* together with *Rhipicephalus appendiculatus* and *Rhipicephalus decoloratus* can potentially transmit Lumpy Skin Disease (LSD) virus (Tuppurainen et al., 2011), which is a disease of socio-economic importance in Southern Africa affecting hide quality and leading to cattle mortalities and productivity losses. There has been an increase in the cases of LSD in Zimbabwe over the past years (Department of Veterinary Services, unpublished).

The genus *Hyalomma* is one of the smallest in the family Ixodidae with 30 species (Jongejan and Uilenberg, 2004). Three species (*Hyalomma truncatum, Hyalomma turanicum* and *Hyalomma rufipes*) are the most widespread in Southern Africa particularly in dry areas (Walker, 1991). *Hyalomma truncatum* has a sharp toxin that when released to the blood stream causes a paralysis known as sweating sickness which affects calves and can be fatal (Jongejan and Uilenberg, 2004). *Hyalomma* species also transmit an important viral disease in humans known as Crimean Congo Haemorrhagic Fever (Gonzalez et al., 1992). Their large mouthparts also result in extensive damage of the skin and other body parts leading to the formation of abscesses, sloughing of teats, lameness, footrot and opened up wounds may be a passage of entry for bacterial pathogens (Walker, 1991). The genus *Ixodes* is the second largest genus in southern Africa with 20 species known (Walker, 1991) and the largest in the world with 241 described species (Jongejan and Uilenberg, 2004). The most important tick species in the region is *Ixodes rubicundus* (karoo paralysis tick) and this tick secretes a toxin that causes paralysis in domestic animals especially in sheep. This tick species is confined to South Africa.

The genus *Haemaphysalis* is the second largest in the family Ixodidae with 168 species (Jongejan and Uilenberg, 2004) of which 10 are found in Southern Africa (Walker, 1991). The most important species in this genus is *Haemaphysalis elliptica,* the South African yellow dog tick (previously referred to as *Haemaphysalis leachi*), known to transmit the most virulent *Babesia canis rossi* causing biliary fever in dogs (Uilenberg, 2006) and also vectors *Rickettsia conori* causing tick bite fever in humans (Beati et al., 1995).

2.1.1. Identification of ixodid ticks

It is important to be able to identify ticks parasitising cattle or other host species as this helps in the diagnosis and control of tick-borne diseases. Identification keys have been developed which help acarologists to morphologically identify tick species (Walker et al., 2003). The family Ixodidae is commonly referred to as the hard ticks because of the dorsal scutum (shield) which covers the dorsum in male ticks while in female ticks it is less pronounced and becomes distended when engorged. The most frequently used features in identification are the mouthparts (hypostome dentition), scutum, adanal plates (for male ticks). Generally male ticks are easier to identify than female ticks, with the latter being much more difficult to identify when they are engorged. The morphological features of male and female hard ticks are shown in **Figure 2-3** and **Figure 2-4**.

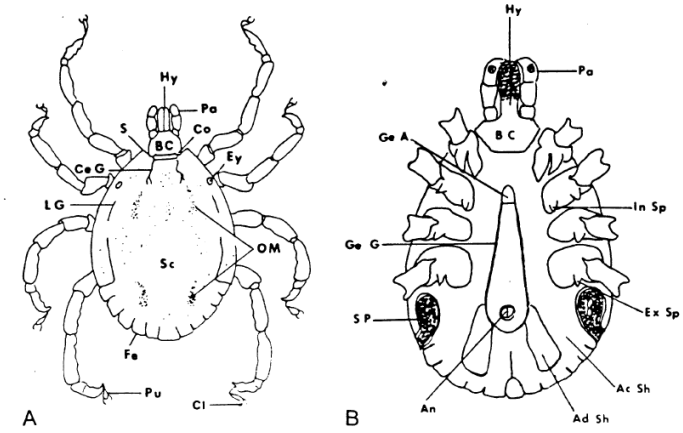


Figure 2-3: A hypothetical adult male ixodid tick, indicating key morphological characters. A, dorsal view: Sc= scutum, BC = basic capituli, Hy= hypostome, Pa=palpus, Co=cornua, Ey= eye, OM = ornate markings, Pu=pulvillus, CL= claw, Fe=festoon, LG = lateral groove, Ce = cervical groove, S=scapula; B, ventral view, BC= basic capituli, Hy = hypostome, Pa= palpus, In Sp = internal spur, Ex Sp = external spur, Ac Sh = accessory shield, Ad Sh = ad-anal shield, An= anus, SP= spiracular plate, Ge G = genital groove, Ge A= genital aperture (Cupp, 1991)

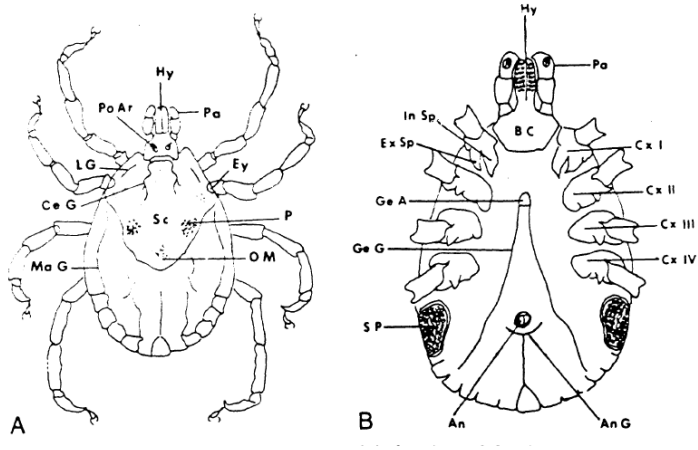


Figure 2-4: A Dorsal view of a hypothetical adult female ixodid tick with key morphological characters labelled as follows, Sc= scutum, PoA =porose areas, Hy= hypostome, Pa= palpus, Ey =eye, P= punctations, OM =ornate markings, Ma G =marginal groove, Ce G = cervical groove, LG =lateral groove B, ventral view BC = basic capituli, Hy =hypostome, Pa= palpus, Cx1 =coxa 1, CxII= coxa II, CxIII= coxa III, coxIV=coxa IV, An G = anal grove, An =anus, SP =spiracular plate, Ge G= genital groove, Ge A=genital aperture, Ex SP=external spur, In Sp=internal spur (Cupp, 1991)

Morphological identification is normally carried out using a stereo-microscope where large ixodid genera such as *Amblyomma* and *Hyalomma* can be easily classified to species level whilst for the smaller rhipicephaline genera a compound microscope is necessary in order to be able to identify the ticks up to species level. The geographic area, host species, and tick attachment site may aid in tick identification. However with the witnessed cattle movement information on known geographic areas may not be of help, hence it is important to always be meticulous in verifying the identity of a tick species using morphological data (Walker et al., 2003). To complement morphological identification, molecular tools have been developed which make use of variable genetic markers such as the Internal Transcribed Spacers (ITS 1 and 2) in the ribosome and Cytochrome oxidases (COX 1 and 2) in the mitochondria (Cruickshank, 2002). These genetic markers are also used to unravel the phylogeny and the genetic linkages of tick species (Navajas and Fenton, 2000). The ITS2 has been useful in verifying the morphological identification of the four boophilid species in areas where they co-exist and where collected tick specimens have been damaged, especially at their mouthparts (Lempereur et al., 2010).

2.2. Distribution of ixodid ticks in southern Africa

Biotic and abiotic factors play an important role in the spread, survival and establishment of ticks to and within different areas (Randolph, 2010). Biotic factors relate to the presence of hosts and vegetation whilst abiotic factors involve the micro- and macroclimate and anthropogenic processes such as habitat modifications, tick control programmes and cattle importation. The distribution of ticks is not static (Tønnesen et al., 2004), hence it is important to always monitor if there have been changes in their distribution which might have influences on the epidemiology of the diseases that they transmit.

Microclimatic factors such as saturation deficit, soil moisture, ground temperature and humidity influence the survival of the developmental stages of tick species (Pfäffle et al., 2013) . Two- and three-host tick species are largely affected by these factors since they spend 99% of their time off the host (Needham and Teel, 1991) only being present on the animals for feeding purposes. Temperature and humidity also influence the establishment of tick species in an area. This has been observed for *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis* in Southern Africa. The former will occupy cool and wetter areas whilst the latter will be present largely in hot and dry areas (Perry et al., 1991b). Another good example is that of *R. microplus* and *R. decoloratus*; as already highlighted the latter is indigenous to the African continent whilst the former has been introduced. *Rhipicephalus microplus* has an invasive character and will displace *R. decoloratus* in areas with warm temperatures and high humidity (Zeman and Lynen, 2010). This displacement has been observed in South Africa, Zambia and Zimbabwe in the early 1980s (Berkvens et al., 1998; Norval et al., 1983; Nyangiwe et al., 2013a). In such areas *R. decoloratus* has been forced to seek for alternative hosts such as wildlife and some have been collected on dogs. However the situation is different in cold dry areas where *R. decoloratus* is still dominant and *R. microplus* fails to displace the former. This has been observed in Tanzania as well (Lynen et al., 2008). In Zimbabwe, it was observed that the distribution of *Amblyomma hebraeum* was limited to the Low and Middle veld and the tick species could not survive in the eastern Highveld due to the cold temperatures which predominate in the area and are not ideal for the survival of the developmental stages (Norval et al., 1994).

The availability of suitable hosts is important as it provides the obligate parasites with blood meals for their survival and proliferation. In Zimbabwe it has been observed that the tick species *Amblyomma variegatum* fails to establish itself in areas whose climate is deemed suitable for the survival because it only infests cattle and buffalo, whilst *Amblyomma hebraeum* is more widespread due to its infestation of a variety of wildlife species apart from cattle. It has actually been hypothesised that for these two tick species, climate plays a lesser role in their distribution whereas the presence of hosts does play a crucial role (Norval et al., 1994). Humans through their activities influence tick distribution and establishment. The recent invasion of *R. microplus* into Namibia (Nyangiwe et al., 2013b) could be related to the importation of cattle from South Africa as a way to improve the genetics of local populations. This has been observed in West Africa where large Girolando breeds of cattle have been imported from Brazil which at the same time has led to the introduction of *R. microplus* (Madder et al., 2007).

Tick control strategies can influence the establishment of tick species in an area. Apart from unfavourable climatic conditions it was observed that the failure of *A. hebraeum* to establish in the eastern Highveld in pre-independence Zimbabwe could be attributed to strong dipping regimes (strict short-interval dipping) being practised at that time (Norval, 1983). However, intensive control programmes broke down during the civil war and could not be fully restored after the war. This situation could have led to the establishment of the tick in these areas after the country's independence (Peter et al., 1998a) . The distribution of ixodid ticks can be influenced by the habitat characteristics such as canopy cover, type of forests and the Normalised Difference Vegetation Index (NDVI) (Pfäffle et al., 2013). These habitat traits will have a significant bearing on the microclimate which is essential for tick survival. The NDVI is based on satellite data measuring the photosynthetic activity on the ground and significantly influences tick abundance in an area (Randolph, 2010). The distribution of *R. appendiculatus* in Kenya has been found to be strongly correlated with NDVI values (Perry et al., 1991a). The latter researchers also reported that overgrazed communal areas in Zimbabwe had a reduced abundance of *R. appendiculatus* which tends to favour savannah and woodland savannah habitats (Norval and Perry, 1990).

Parapatric (abutting and marginally overlapping distributions), sympatric (sharing the same location) and allopatric (separated and non-abutting distributions) relationships amongst ixodid ticks can influence their distribution. The distribution of *A. variegatum* had a southern limit in Zimbabwe in the 1930s while that of *A. hebraeum* had a northern limit and these two species where allopatric. Over the next three decades, however, the two *Amblyomma* species were found to be parapatrically distributed (Norval, 1983). Parapatric distribution of the two *Amblyomma* species has also been recently reported in Mozambique (Bournez et al., 2015). Parapatric distribution has also been observed for *R. microplus* and *R. decoloratus* in Tanzania (Lynen et al., 2008) where the two species reach equilibrium at a climatic gradient of 22-23°C isotherm and 58mm isohyet. This limits the expansion of either species and creates a zone of overlap where the two species interact. In both these cases (*Amblyomma* and boophilids) parapatry has been attributed to the exclusive competition between two species in contact zones for hosts, attachment sites and sexual mates (reproductive interference).

2.3. Predictive modelling of tick distributions

The advent of geographic information systems (GIS) and remote sensing technologies has enabled the prediction of species distribution by taking into account their habitat, climatic and ecological niches (Hirzel and Le Lay, 2008). The presence of occurrence data for a species, environmental predictor variables and use of appropriate statistical models will enable us to estimate the distribution of a particular species (Hijmans and Elith, 2016), computing the probability of occurrence in areas not yet sampled (De Clercq et al., 2013) and provide information on how it could be affected by changes in the environment (Randolph, 2000).

Since climate is the most limiting factor of ixodid tick distribution (Cumming, 2002), interpolated world climatic data sets (e.g. WorldClim) have been useful in estimating species distribution (Estrada-Peña et al., 2016). These datasets are based on average weather data collected at meteorological stations from local to global scales (De Clercq et al., 2015). The datasets are then used together with some statistical framework (usually logistic regression) and observed tick distribution to deduce the likely geographic range of a tick species which is essentially a pattern matching procedure

(Lèger et al., 2013). Other procedures involve the understanding of the biological processes (process based models) of the ixodid ticks and how these are influenced by climate. These are rarely used due to the lack of knowledge on the drivers of physiological process for many tick species (Estrada-Peña et al., 2016). The pattern matching methods though not entirely accurate are the most common methods used in predictive modelling. The estimation of the distribution of ixodid tick species will help policy makers in coming up with effective vector control programmes which entail monitoring invasive tick species and simultaneously the pathogens that they transmit.

Predictive models for ixodid tick distribution can use climatic (temperature and water) as well as vegetation indices as variables to estimate the distribution of species. CLIMEX is the first package to be used for predicting tick distribution. This model uses temperature and water indices (hot, cold, wet and dry) (Sutherst and Maywald, 1985) and was used to predict the distribution of *Boophilus microplus* (now *Rhipicephalus australis*) in Australia and in sub-Saharan Africa (now *R. microplus*), *R. appendiculatus* (Perry et al., 1991a) as well as *Amblyomma variegatum* and *A. hebraeum* in Africa (Norval et al., 1991c; Perry et al., 1991b). Information from weather stations around Africa was used to develop an interpolated climate dataset at a resolution of 25km². This dataset was used to run a climate-matching model which calculates an eco-climatic index at a scale of 1-100, predicting the likely suitability of the environment for the tick. The model overestimated the distribution of *R. microplus* and predicted that this tick species had a suitable habitat in West Africa although the tick was not found in the region until two decades later (Madder et al., 2007). The predicted habitat suitability of the *Amblyomma* species was opposite to the situation prevailing on the ground. The use of a four point algorithm (monthly maximum and minimum temperature, rainfall and evaporation) could have influenced these results as these did not take seasonality into account (De Clercq et al., 2013).

Another package mostly used in species distribution modelling is BIOCLIM whose first use dates back to the early 1980s (Booth et al., 2014). The utility of the package has replaced that of CLIMEX over the years. BIOCLIM uses 19 climatic variables based on maximum, minimum and mean values of temperature and rainfall (monthly, quarterly and annually). This package together with a vegetation index (NDVI) has been useful in predicting the distribution of ixodid tick species throughout the world and how the

current distributions will be influenced by climate change (Randolph, 2000). The NDVI is a measure indicating the available humidity within the vegetation layer on the ground which is essential for tick survival during the developmental stages (Estrada-Peña et al., 2016). The annual NDVI has been found to be a better predictor of tick distributions than the annual means for temperature and rainfall (both minimum and maximum) (Cumming, 2002). Other packages have been developed to predict habitat suitability (Guisan and Thuiller, 2005) see Table 2-1.

Table 2-1: Some of the predictive species distribution models in use (Guisan and Thuiller, 2005)

Tool	Methods Implemented
BIOCLIM	Climatic Envelope
ANNUCLIM	Climatic Envelope
BAYES	Bayesian approach
BIOMAPPER	Ecological Niche Factor Analysis
BIOMOD	Generalised Linear Model, Generalised Additive Models, Classification and Regression Trees, Artificial Neural Networks
DIVA	Climatic Envelope
DOMAIN	Climatic Envelope
ECOSPAT	Generalised Linear Model, Generalised Additive Model
GARP	Genetic Algorithm
GDM	Generalised Dissimilarity Model
GRASP	Generalised Linear Model, Generalised Additive Model
MAXENT	Maximum Entropy
SPECIES	Artificial Neural Networks

Apart from using interpolated climatic data sets, remotely sensed data can also be used. Examples which have been used are the Advanced Very High Resolution Radiometer (AVHRRI) (Olwoch et al., 2007) run by the National Oceanic and Atmospheric Administration (NOAA) meteorological satellites and the Moderate Resolution Imaging Spectroradiometer (MODIS) (De Clercq et al., 2015). These offer an added advantage as the variables so used are more reduced and less prone to collinearity as compared to BIOCLIM data (Estrada-Peña et al., 2016). Furthermore remote sensing enhances the feasibility of collecting data in areas deemed technically difficult to access (Rushton et al., 2004). The AVHRRI uses satellite derived monthly NDVI as the main predictor of tick distribution while MODIS uses satellite derived data on daytime land surface temperature (dLST) and NDVI. De Clercq et al. (2015) found out that results with MODIS (remote sensing data) derived variables provided better accuracy than those from WorldClim data (interpolated dataset).

Using predictive models, the ecological preferences of the ticks of the subgenus *Boophilus* (*R. microplus*, *R. geigyi*, *R. decoloratus* and *R. annulatus*) have been studied for Africa and Latin America (De Clercq et al., 2015; Estrada-Peña et al., 2006a; Estrada-Peña et al., 2006b). *Rhipicephalus geigyi* and *R. annulatus* have been collected in areas of high temperature and rainfall and they are most likely to appear together as they share the same signatures of NDVI in western Africa (Estrada-Peña et al., 2006a). The results showed that *R. decoloratus* is located mostly in areas of low temperature and rainfall. *Rhipicephalus microplus* prefers high rainfall areas and can withstand long periods of drought although it is located within the n-dimensional ecological niche of demes of *R. decoloratus*. The ecological preferences of *R. microplus* populations from America and Africa are so different that locations in one continent cannot be used to extrapolate what could happen in another continent. As far as the predicted distributional ranges of ixodid tick species have been studied, these have been found to vary from study to study as more records become available and more robust statistical models are developed (De Clercq et al., 2013).

2.4. Population genetics of ixodid ticks

In population genetics, the frequency and interactions of alleles in a population is investigated and this is crucial in answering many questions in ecology where the role of dispersal or gene flow has to be explained (Kim and Sappington, 2013). Genetic approaches have become important in our understanding of ecological and biological processes over the years (Selkoe and Toonen, 2006). This is more so in the ecological studies of vectors which are important as biological and mechanical transmitters of disease pathogens in humans and animals worldwide (Jongejan and Uilenberg, 2004) . Vector ecology involves understanding the factors that influence the movement, establishment and adaptation of these organisms to the environment and hosts (Chevillon et al., 2007). Transmission patterns, migration rates, isolation by distance, genetic relatedness, genetic diversity, population assignment, bottlenecks, effective population size determination, paternity testing, invasion biology, routes of invasion and resistance management can also be explored through genetic studies (De Meeùs et al., 2010; Kim and Sappington, 2013; Weising et al., 2005). Some of these parameters are very difficult to measure in small organisms like ticks and the use of genetic markers such as allozymes, microsatellites, mitochondrial and nuclear DNA sequences will provide a platform by which these can be quantified (McCoy, 2008). Knowledge of such information particularly in vector ecology will be useful in the understanding of disease epidemiology as well as coming up with improved vector control strategies (Tabachnick and Black IV, 1995).

Of the genetic markers previously mentioned, microsatellites are the most informative (Hoshino et al., 2012), they have a high mutation rate, belong to a class of molecular markers that are Polymerase Chain Reaction (PCR) based, co-dominant and highly polymorphic, these factors make them ideal for studying evolution and genetic variation in organisms (Jarne and Lagoda, 1996). Microsatellites which can also be referred to as simple sequence repeats (SSRs) are tandemly repeated tracts of DNA composed of 1-6 base pair (bp) long units being found widely distributed both in eukaryotes and prokaryotic genomes in coding and non-coding regions (Tóth et al., 2000).

A comprehensive review on tick population genetic studies dating a period of 30 years is given by Araya-Anchetta et al. (2015). The review focus on works done at a local and large scale on Argasidae and Ixodidae ticks infesting a wide range of hosts. This together with a review by McCoy (2008) detail the importance of studying the population genetics of vectors with examples given on the following species; *Ornithodoros*, *Amblyomma*, *Dermacentor*, *Ixodes* and the *Rhipicephalus (Boophilus)* ticks . These studies have provided valuable insight on the transmission ecology of the host parasite system, evolution of host specificity and reciprocal adaptations in host-tick interactions as well as evolutionary forces driving resistance to chemical acaricides. Despite this, the population genetic structure of most tick species remains relatively unknown with most attention being given to the pathogens (Van Houtte et al., 2013). Further, it is noticed that most of these studies have been carried out in Europe, America and Australia. Only recently that a population structure study was done in East Africa on one of the most important ticks; *Rhipicephalus appendiculatus* (Kanduma et al., 2015). There is need to scale up such studies in Africa as knowledge on vector genetics will provide insights on the interactions that occur between vectors and pathogens (Gooding, 1996) providing better understanding of tick-borne disease epidemiology.

2.5. Participatory epidemiology

Over the years in many developing countries there has been a shift in the funding of tick control programmes from government subsidised to a situation where farmers are responsible for providing funds for tick control (Peter et al., 2005). This has been largely caused by economic structural adjustment programmes which have transferred the financial burden of tick control to farmers with governments playing a co-coordinative role (Rich and Perry, 2012). Resource-poor communal farmers now play a pivotal role in as far as tick control is concerned. These farmers can be made to organise themselves and wholly superintend their tick control programmes. Livestock Development Committees (LDCs) have been set up which are responsible for co-ordinating animal health management activities in their communities with the assistance of the Veterinary Services Departments. As has already been highlighted, in communal settings the plunge dip is the most common way by which acaricides are applied on cattle. These plunge dips need to be filled with water, they need to be maintained so that they do not

silt and they have to be replenished time and again. This is where these committees come into play.

Farmers can also be a source of information on the epidemiology of diseases affecting an area, through their interaction with animals on a daily basis they can be in a position to well articulate disease problems affecting their livestock (Mariner et al., 2011). They can do this in their own traditional ways using local languages and synonyms for diseases, giving clinical presentations, epidemiological patterns and simple pathological lesions (Mugisha et al., 2008). Such information is useful in the scientific domain in terms of understanding disease epidemiology in such situations. This can offset the costs that researchers and governments have to incur in setting up and conducting surveillance programmes and conventional studies of the epidemiology of livestock diseases. It has been observed that although farmers may not have intricate scientific knowledge about a disease, their indigenous knowledge will offer a wealth of information that will be helpful in understanding the epidemiology of a disease (Grace et al., 2008). Thus a new branch of epidemiology has been growing over the years which seeks to involve cattle owners in identifying their own problems, how the problems could have been created and potential ways of solving them. This is referred to as participatory epidemiology (PE) (Catley et al., 2012). Participatory epidemiological studies have been carried out in several countries to understand farmers' perceptions, attitudes and knowledge of diseases affecting their livestock. Information obtained from such studies has gone a long way in implementing effective disease control programmes. Participatory epidemiology studies may involve conducting informal interviews (focus group discussions and semi-structured questionnaires), use of seasonal calendars (history and timing of disease events), ranking and scoring (matrix or simple scoring). This can be a quick and cheap way of data collection. This is a deviation from conventional research approaches, although the latter may be used in a triangulation approach to validate the results from participatory surveys. Participatory studies on ticks and TBDs have been conducted in different parts of Africa (Catley and Aden, 1996; Chatikobo et al., 2009; Chenyambuga et al., 2010; Hlatshwayo and Mbati, 2005; Masika et al., 1997; Mugisha et al., 2005). In most of the cases, farmers were able to relate the most common ticks in the area, their effects on livestock and the period in which they are abundant on cattle and the environment. Formal questionnaires which involve structured questions can also be used providing a wealth of information useful for tick

management programmes. Animal disease control programmes have shown to be useful when farmers input is solicited. A top-down approach where policy makers and veterinary authorities impose decisions on farmers has yielded very poor results (Catley et al., 2012).

2.6. Acaricides in the control of cattle ticks

The control of ticks has largely relied on the use of chemicals known as acaricides (Jongejan and Uilenberg, 1994), despite the availability of other potentially effective methods (George, 2000). The characteristic of a good acaricide is one which will kill the tick, does not harm the animal or the applicator, does not leave residues in the tissues of animals treated and has no adverse effects to the environment (Graf et al., 2004). The first acaricide to be used to control ticks late in the 19^{th} century was arsenic made from a water soluble compound sodium arsenite (As_2O_3) (George et al., 2004). The effectiveness of this acaricide led to its intensive use in several parts of the world over a period of five decades. Since the 1940s, there has been a series of chemical acaricides developed for tick control starting with organochlorines followed by organophosphates, carbamates, formamidines, synthetic pyrethroids and macrocyclic lactones. The development of new acaricides was necessitated by issues to do with toxicity to not only ticks but the vertebrates, presence of residues in meat after slaughter of animals, environmental issues and development of resistance by ticks (George et al., 2004). The formamidines are currently one of the most extensively used acaricides in the world (Jonsson and Hope, 2007).

A new class of ectoparasiticides called isoxazolines have been developed and have been evaluated mostly in companion animals (Pfister and Armstrong, 2016). They are administered orally and can offer protection for up to 4 weeks and have been found to have a faster onset of action (McTier et al., 2016). The traditional class of acaricides are administered topically and will be distributed cutaneously on the skin surface while the orally administered isoxazolines are distributed systemically (Pfister and Armstrong, 2016). The latter will quickly and widely distribute in blood circulation being able to kill attached ticks at any part of the body the tick feeds. This immediate acaricidal efficacy will reduce tick attachment time and consequently the risk of pathogen transmission (Burgio et al., 2016). Despite their fast onset of action, the long residual effect these

drugs have might have negative implications on human health especially if the withdrawal period are not taken into account.

2.6.1. Mechanism of action of common acaricides used to control cattle ticks

Traditionally the veterinary acaricide market has relied on reformulations of compounds developed and tested for crop protection (Taylor, 2001). Much of what is known by the mode of action of these acaricides has been derived from studies conducted on other arthropods such as insects and mites (Guerrero et al., 2012). The knowledge on the mode of action of acaricides will be important in allowing to screen for target site resistant alleles, leading to the development of diagnostic tools which will be helpful in monitoring resistance management (Van Leeuwen et al., 2010). Most acaricides in use for tick control in cattle are neurotoxins and exert their effects on the arthropod's nervous system (Taylor, 2001). The acaricides commonly used to control ticks can be classified according to their mode of action as; acetyl choline esterase inhibitors, GABA-gated chloride channel antagonists, sodium channel modulators, glutamate-gated chloride channel allosteric modulators and the formamidines (Sparks and Nauen, 2015).

Acetylcholine esterase inhibitors are the organophosphates (OPs) and carbamates and their mechanism of action in insects has been reviewed extensively by Fukuto (1990). Organophosphates act by inhibiting the action of the enzyme Acetylcholine (AChe), through irreversible transphosphorylation of the enzyme making it unable to breakdown the neurotransmitter acetylcholine, this results in continuous sending of nerve signals and neuromuscular paralysis of the arthropod. Carbamates' mode of action towards the enzyme acetylcholine esterase is slightly different as they appear to cause a spontaneously reversible block on the AChe enzyme. Apart from resistance development, another factor impeding the use of these AChe inhibitors is that for instance, carbarly (a carbamate) has been found to be carcinogenic while OPs can be extremely toxic to humans and animals and their degradation in the environment is slow taking up to six weeks (George et al., 2004). A new group of acaricides the spinosyns (Ware, 2000) activates nicotinic acetylcholine receptors and secondarily antagonises GABA receptors , a member of this group spinosad has been found effective against the

nymphal and larval stages of *Rhipicephalus microplus* (Davey et al., 2001) and has been used in rotation programmes with amitraz (Jonsson et al., 2010b).

Another group of acaricides are the GABA-gated chloride channel antagonists, in this category are the organochlorines (hexachlorohexanes), milbemycins (Casida, 1993) and phenyl pyrazoles (Cole et al., 1993). These block the transmission of signals by the neurotransmitter gamma amino butyric acid (GABA), which is an inhibitor, the compound binds within the chloride channel and consequently inhibits the influx of chloride ions into the nerve cell causing hyper excitation and paralysis of the arthropod nervous system. The use of organochlorines in treating livestock has declined due to their persistence in the environment and toxicity issues and they have been withdrawn from the market (Kunz and Kemp, 1994). Pyrethroids and the organochlorine Dichlorodiphenyltrichloroethane (DDT) are grouped as sodium channel modulators (Davies et al., 2007). These target voltage-gated sodium channels which are transmembrane proteins responsible for electric signalling and propagation of action potentials (Dong, 2007). When the pyrethroids and DDT are applied on ticks, they keep the sodium channels open which increases nerve membrane permeabilities to sodium ions leading to the paralysis and death of the parasite (Davies et al., 2007) (Figure 2-7).

Closely related to the mode of action of the GABA gated chloride channel antagonists are the macrocyclic lactones (avermectins) known as the glutamate-gated chloride channel allosteric modulators (Nauen and Bretschneider, 2002). These compounds apart from being useful as acaricides are active against a wide range of nematodes and because of their toxic action on internal and external parasites they are referred to as endoctocides (Shoop et al., 1995). The mode of action of these compounds is that they act on the gamma-aminobutyric acid (GABA) blocking stimulation of excitatory motor neurones resulting in flaccid paralysis (Bloomquist, 1993). The high cost of macrocyclic lactones overrides their efficacy thus limiting their use in tick control (Kemp et al., 1999). The formamidines of which the main member is amitraz are referred to as octopamine receptor agonists. Amitraz is the only formamidine approved for the control of ectoparasites (De Meneghi et al, 2016). The main target of amitraz is thought to be the octopamine receptor in the central nervous system of arthropod species (Evans and Gee, 1980), this results in neuronal hyper excitability and death of the arthropod (Taylor, 2001). More information on other acaricides can be found in Table 2-2. The diagram in

Figure 2-5 shows two nerve cells separated by a synapse with the different regions of the nerve cell illustrated (A) and the target sites of the different chemicals (B).

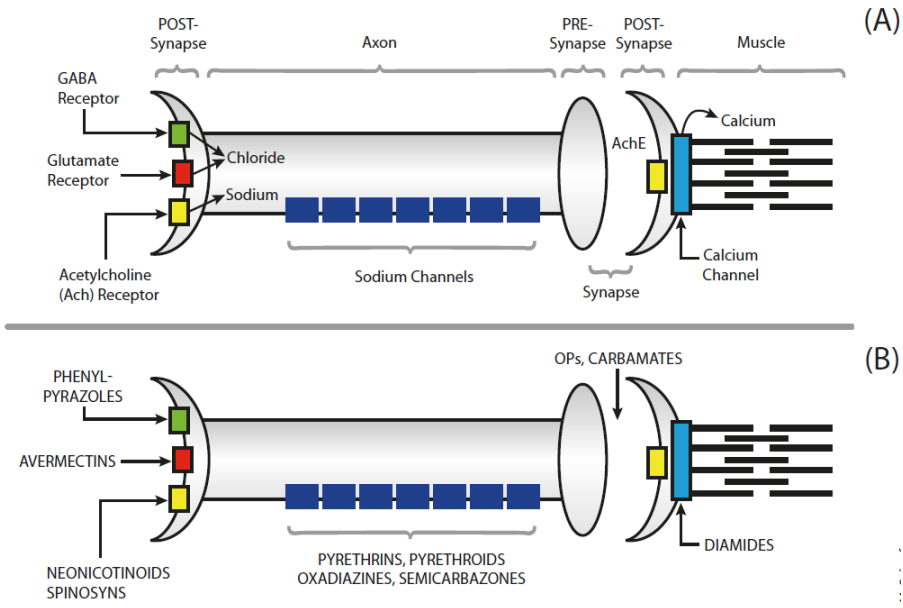


Figure 2-5: Neurological target sites of commonly used acaricides (adapted from: Suiter and Scharf, 2009)

There are other chemicals which are used to control insects and mites and these target the mitochondrial respiration process and inhibit insect or mite growth and development (Sparks and Nauen, 2015). In light of the emergence of resistance in cattle ticks , there is need to develop novel mode of action compounds, but this is hampered by the fact that most of these acaricides target the nervous system and there is a limit to the number of neuro active target sites, research focusing on compounds that target growth regulators is increasingly becoming important (Taylor, 2001).

Table 2-2: Mode of action and target sites for commonly used acaricides for tick control in cattle (Adapted from Taylor, 2001)

Target function	Receptors	Chemical Class	Compounds
Neurotransmission	GABA glutamate gated Chloride channels	Avermectins	Ivermectin, doramectin
	GABA gated chloride channels	Milbemycins	Moxidectin,
		Phenylpyrazoles	Milbemycin oxime,
		Hexachlorocyclohexanes (Organochlorines)	Fipronil Benzene Hexane Chloride (BHC)
	Acetylcholine esterase (AChe)	Organophosphates	Coumaphos, chlorfenvinphos, diazinon, malathion
	Acetylcholine (nicotinic receptors)	Carbamates	Carbaryl, propoxur
	Sodium Channels	Pyrethroids	Deltamethrin, permethrin, cypermethrin, flumethrin,
		Chlorinated ethanes	
	Calcium Channels	Cyclodienes (Organochlorine)	Dieldrin, aldrin
	Octopamine receptors	Amidines	Amitraz
Enzyme Inhibition	Mixed function Oxidases	Methylymedioxphenyl compounds	Piperonyl butoxide
Moulting	Chitin synthesis inhibitors	Benzylphenoyl ureas	Fluazuron
	Chitin inhibitors	Triazine derivatives	Cyromazine
		Pyrimidine derivatives	Dicyclanil
	Juvenile hormone analogues	Terpenoid compounds	Methoprene
		Carbamates	Fenoxycarb

2.6.2. Application of acaricides on cattle

Acaricides can be applied on cattle in a number of ways. The traditional means of application is through the use of a plunge dip (Figure 2-6) and spraying (using a spray race or handsprayers) (Jongejan and Uilenberg, 1994). New formulations such as pour-ons, hand dressing, intra-ruminal boluses, injectables, neck bands and acaricide impregnated ear tags have also been introduced (George, 2000). The plunge dip is a very common application method both in resource poor and commercial settings and is largely referred to as “dipping vats” in the Americas and Australia (Angus, 1996; George, 2000). A dip is constructed and filled with water (can be up to 10 000 litres of water) where after an acaricide is added to yield a specific concentration. Then, a group of about 30 cattle enters the dip to mix the acaricide with water. These animals should re-enter the dip again when the session is in swing. The advantage of the plunge dip is that it ensures whole body contact with the acaricide but dairy animals with larger udders can be injured during the dipping process (Young et al., 1988).

A spray race (Figure 2-6) is used largely by commercial dairy farmers to circumvent the issues with the plunge dip. Here, cattle pass through a race or corridor where they are sprayed with a diluted acaricide chemical. In both these cases, a large number of animals can be treated with acaricides over a relatively short period of time but the spray races can be very costly to construct, hand spraying (Figure 2-6) pour-on and hand dressing methods are useful when tick control is targeted at low numbers of cattle as they can be labour intensive and also a lot of acaricide is wasted during the procedure (Wilson, 1996). The handspraying and pour-on methods may not target all the tick predilection sites. Hand dressing is targeted at applying acaricides (mostly pyrethroids as tick grease) by hand to areas where the dipping or spraying methods cannot effectively kill the ticks; these are the inner parts of the ear, under parts of the tail, tail brush, perineum and between the teats. Methods such as the use of impregnated ear tags as slow release devices offer advantages in that they have a long residual effect (George, 2000). In Kenya, an intra-ruminal ivermectin slow-release device which offers 90 day protection against tick damage (Pegram et al., 1993) and a mechanical Duncan applicator (Figure 2-6) have been found useful in game reserves (Duncan, 1992).

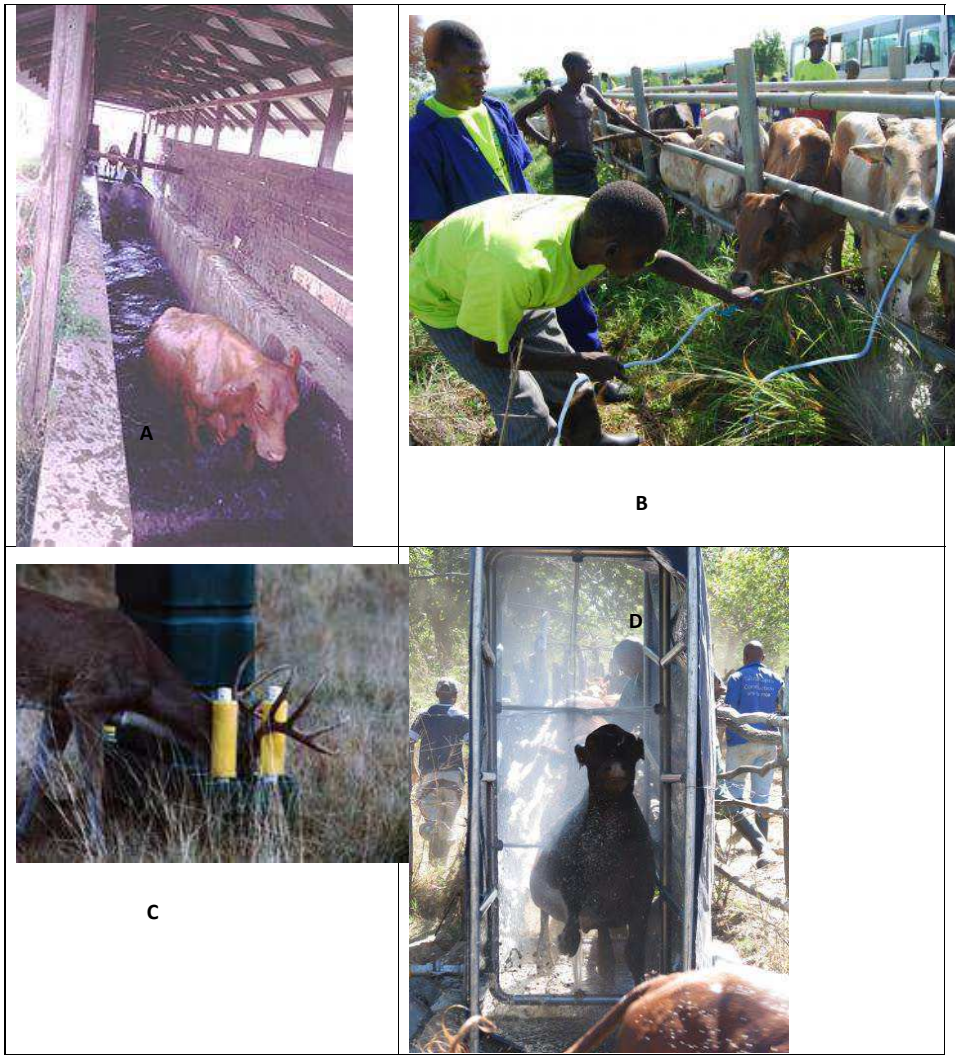


Figure 2-6: Illustrations of acaricide application methods. (A) Plunge dip, (B) Hand spraying (C) Duncan Applicator (D) Spray race ; Source; (Latif and Walker, 2004)

2.6.3. Factors influencing frequency of acaricide application

The frequency of use of acaricides will depend on the epidemiology of tick borne diseases in the area, tick abundance and the objective (eradication or control). Tick-borne diseases may occur as epidemics (outbreaks) affecting a large number of animals at once, for instance when a tick species has invaded a susceptible population or when the latter is introduced in a tick infested area (Uilenberg, 1976). An example of an outbreak occurred in 1900 when Cecil John Rhodes imported 1000 cattle from Australia to the then Southern Rhodesia. All but 3 of the imported cattle died suspectedly due to TBDs to which they had never been exposed to before (Norval, 1985a). An introduction of the tick *R. microplus* in an area may lead to outbreaks of bovine babesiosis caused by *Babesia bovis* and this may result in cattle mortalities in unvaccinated animals or those not previously exposed to the disease. In such kind of situations absolute or intensive control of ticks with the aim of eradication can be applied, as has occurred in the southern United States of America during the eradication of *R. microplus*.

Endemic situations may be observed, these are characterised by the frequent seasonal occurrence of a disease (Uilenberg, 1976). Tick-borne diseases are endemic in areas where vector ticks are found but they can be stable or unstable endemics (Bezuidenhout, 1985). Stable endemic situations arise when there is co-evolution and adaptation amongst the vector-host-pathogen relationship such that the disease frequently occurs without any mortalities being recorded and in some cases clinical signs being in apparent (Peter et al., 2005). Babesiosis, anaplasmosis and to some extent cowdriosis are endemically stable TBDs, while this is rarely observed for theileriosis. In endemically stable situations tick control can be done at predetermined commencement dates and regular intervals regardless of the number of ticks present. As such, governments such as in Zimbabwe have adopted a weekly interval dipping during the rainy season when tick numbers are high and a fortnight dipping interval during the dry season when tick numbers are low. This is largely referred to as strategic tick control (Wilson, 1996).

Unstable endemic situations on the other hand are characterised by the frequent occurrence of disease accompanied by mortalities in young animals, which is common

in *Theileria parva* infections (Norval et al., 1992b). In order to promote stability, for endemically unstable situations, the aim should be to maintain tick numbers at levels which will not cause disease; hence, prophylactic or threshold treatment of animals can be done (Pegram et al., 1993). This will involve reducing the number of ticks to acceptable levels and determining the maximum number of ticks upon which treatment should be done. Intensive or absolute use of acaricides may create unstable situations when there is no consistency in the programmes as in the Zimbabwe civil war of the late 1970s (Pegram et al., 2000). There was a breakdown of dipping services and since most of the cattle were not immune protected (stable endemic), close to a million cattle are reported to have died as a result (Norval et al., 1991b). Integrated tick control measures will incorporate all these strategies as well as biological means (e.g. use of tick resistance breeds) and the use of anti-tick vaccines and vaccines for TBDs (Jongejan and Uilenberg, 1994).

2.6.4. Acaricide resistance in ixodid ticks

Acaricide resistance is a genetic condition that confers in a tick population, the capability of an adaptation, to succeed before a toxic environment, promoted either naturally or artificially (Rosario-Cruz et al., 2009). An acaricide per se is not the causative agent of resistance, but through intensive use, favourable mutations inherent in the population are selected with susceptible individuals being eliminated and those with the mutant allele being established in the population (Corley et al., 2013).

2.6.4.1. Development of acaricide resistance in ixodid ticks

As far as ixodid ticks are concerned, much effort has been put into the study of mechanisms of resistance especially for the one host tick *R. microplus*, largely due to its undisputed global importance (Willadsen, 2006). Acaricide resistance remains the single biggest hindrance in the control of vector-borne diseases (Rosario-Cruz et al, 2009). Metabolic detoxification and changes in the target site are the main mechanism by which ticks become resistant to acaricides although reduced penetration has been described (Guerrero et al, 2012).

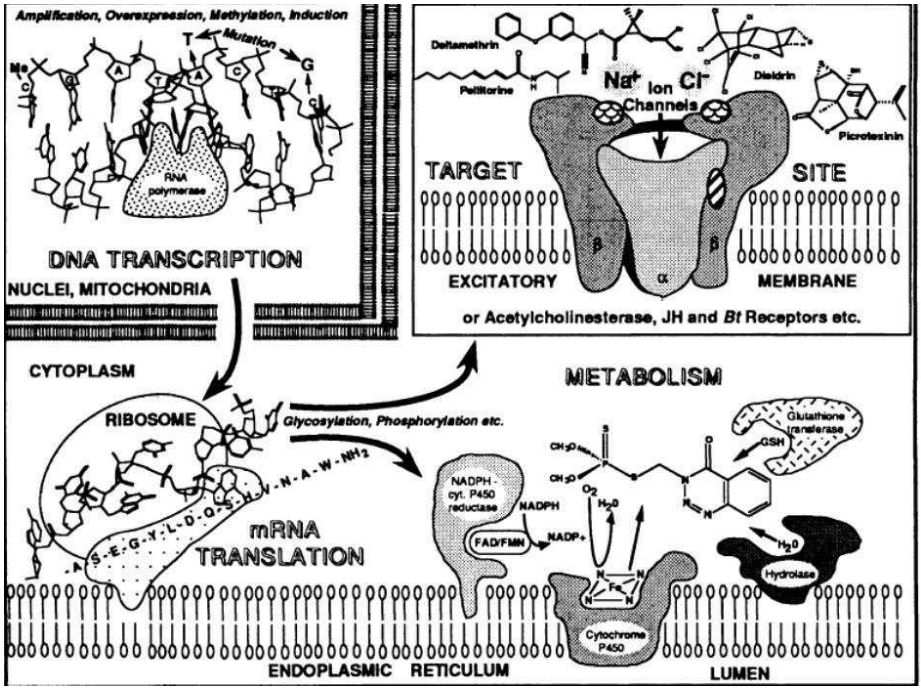


Figure 2-7: Diagram showing transcription and translation of genes from DNA to protein conferring target site or metabolic detoxification resistance and their position within a cell, JH-Juvenile Hormone, Bt-Bacillus thuringeiensis (source: Mullin and Scott, 1992)

Metabolic detoxification involves the action of enzymatic systems such as increased metabolism of the arthropods by cytochrome P450 mono-oxygenases (Multi-function-oxidase-MFOs), the increased activity of glutathione S-transferases (GSTs) and the increased hydrolysis or sequestration by esterases (e.g., carboxylesterases) which play an important role in resistance to organophosphates and pyrethroids. Synergist studies using inhibitors such as piperonyl butoxide (PBO), triphenyl phosphate (TPP) and diethyly maelate (DEM) specific for the cytochromes, carboxylesterases and glutathione transeferases respectively have enabled the understanding of metabolic resistance in *R. microplus* ticks and other athropods. However since these enzymatic systems are encoded by large gene families, cytochrome P450 (81), glutathione S transferases (39) and the carboxylesterases (115) it has not been possible to specify a gene-mediated mediated metabolic resistance mechanism in tick species as it has been in insects such as *Anopheles gambiae*, *Musca domestica* and *Drosophila melanogaster* (Guerrero et al., 2012). Evidence of metabolic resistance in *R. microplus* involving the

carboxylesterase (Cae) enzyme was confirmed by Jamroz et al. (2000) who isolated and identified an esterase (CzEst9) whose activity was increased in a pyrethroids resistant Mexican strain ("Coatzacoalcos"-Cz) of *R. microplus*. This was further characterised by Pruett et al. (2002) who concluded that the esterase offered moderate levels of resistance to pyrethroids. Over expression of the esterase provides an incremental and statistically significant additional amount of pyrethroid resistance by hydrolysing the acaricide (Guerrero et al, 2002).

Genes encoding for acetylcholine esterase have been identified in several arthropod species of which three were found in *R. microplus* ticks, BmAChe1, BmAChe2 and BmAChe3 (Baxter and Baker, 1998, Temeyer et al., 2004). Mutations in these genes will result in reduced inhibition of acetylcholine. Pruett (2002) showed that an insensitive acetylcholine esterase was involved in OP resistance. Temeyer et al. (2010) cloned and expressed recombinant the three acetylcholine esterase enzymes which had reduced sensitivity to OP-inhibition from OP-resistant strains. Two of these (rBMAChe1 and rBMAChe3) contained mutations expressed as OP-insensitive enzymes. The results suggested that phenotypic resistance to OPs may be complex and multigenic in nature. Recently Ghosh et al. (2015) identified 4 mutations in BmAChe2 from OP resistant Indian isolates of *R. microplus* which could be associated with resistance.

Mutations leading to structural changes in the genes encoding the detoxifying enzymes such as the carboxylesterase have been identified in *R. microplus* ticks by Hernandez et al. (2000) in the Cz Mexican strain resistant to pyrethroids which resulted in an increased amounts of esterase activity, this mutation G1120A resulted in nucleotide change at position 1120 of the putative carboxylesterase gene that led in an amino acid change from aspartate to asparagine. The same point mutation was found to correlate with a malathion resistant (OP) *R. microplus* strain from Mexico (Baffi et al, 2007). This mutation could not be found in the Australian strain of *R. microplus* (Chen et al., 2009).

Toxicological studies by Miller et al. (1999) using synergists could not establish the role of metabolic resistance in a pyrethroids resistant strains of *R. microplus* from Mexico (Corrales and San Felipe), and they suggested that target site mutations could be involved in causing resistance in these tick populations. He et al. (1999), identified a mutation in the S6 segment of the domain III of the VGS gene in the same pyrethroid

resistant strains (Corrales and San Felipe) of *R. microplus* in Mexico. This mutation (T2134A) result in a phenylalanine to isoleucine amino acid substitution. However this mutation was found to be absent from the Australian, Brazilian and South African strains of *R. microplus* (Lovis et al., 2012). Morgan et al. (2009) and Jonsson et al. (2010a) identified mutations C190A (leucine to iso-leucine amino acid substitution) and G72V (glycine to valine amino acid substitution) respectively in the S4-5 linker of the domain II of the VGS in pyrethroid resistant tick populations from Australia. The C190A mutation was found to have a wide distribution being present in Australia, Brazil, Mexico and South Africa while the G72V mutation appeared restricted to Australia (Lovis et al., 2012). Recently Stone et al. (2014) discovered a novel mutation in pyrethroid resistant *R. microplus* isolates from the United States of America and Mexico. This mutation is located in the domain II of the VGS and that has been found to be associated with extreme levels of resistance (super-knockdown resistance) in other arthropods (Guerrero et al., 1997).

Various mechanisms of resistance to amitraz have been proposed namely metabolic through the action of hydrolysing esterases, Li et al. (2004) found higher TPP (esterase inhibitor) synergism with amitraz toxicity although with PBO (MFO inhibitor) this was variable with synergism both in the susceptible and resistant strains. Synergism of amitraz toxcicity was also found with DEM (GST inhibitors) suggesting that glutathione S transferases could be involved in metabolic resistance to amitraz (Fragoso-Sanchez et al., 2011). Chen et al. (2007) discovered two nucleotide mutations in a putative octopamine receptor gene in amitraz resistant strains from Mexico and Brazil which were absent in susceptible strains. These two mutations were confirmed to be associated with amitraz resistance in *R. microplus* strains from South Africa (Baron et al., 2015). There is also new evidence suggesting that one of the octopamine receptor sites, the beta-adregenic is associated with amitraz resistance in tick strains from Australia (*R. australis*) formerly *R. microplus* (Corley et al., 2013; Koh-Tan et al., 2016).

Table 2-3: Evidence of resistance mechanisms in *R. microplus* ticks to pyrethroids, organophosphates and amitraz.

Acaricide Class	Resistance mechanism	References
Pyrethroids	Target site insensitivity (VGS, domain II and III)	He et al., (1999) ; Guerrero et al., (2001); Rosario-Cruz et al., (2005) ; Morgan et al., (2009) ; Jonsson et al, (2010a) ; Stone et al, (2014)
	Metabolic resistance (carboxylesterase)	Miller et al, (1999), Jamroz et al, (2000) ; Pruett et al, (2002) ; Hernandez et al, (2000)
Organophosphates	Target site insensitivity (AChe)	Temeyer et al., (2010); Ghosh et al., (2015)
	Metabolic resistance (carboxylesterase, cytochrome P450s)	Baffi et al., (2007) ; Li et al., (2003)
Amitraz	Target site insensitivity (octopamine tyramine receptors)	Chen et al., (2007); Chen et al., (2009) ; Baron et al., (2015), Corley et al., (2013), Koh-Tan et al., (2016)
	Metabolic resistance	Li et al., (2004)

Although there has been some attempts in establishing evidence of resistance mechanism in tick species, this cannot be matched with work that has been done in insects and mites probably due to the lower market value of the ectoparasiticdes market as compared to the pesticide market for crop protection (Taylor, 2001). It is important to note that these studies have improved our understanding of the molecular basis behind acaricide resistance in cattle ticks, at the same time creating opportunities for the development of new diagnostic tools for acaricide resistance detection (Guerrero et al., 2012).

2.6.5. Diagnosis of acaricide resistance

2.6.5.1. Bioassays

The diagnosis of acaricide resistance has been traditionally done by the use of bioassays for the past 50 years (George et al., 2004). These bioassays will give the phenotypic status of a tick population in as far as susceptibility to a particular acaricide is concerned (Rosario-Cruz et al., 2009). The first bioassay to be developed was the adult immersion test (AIT) by Drummond et al., (1973) which has been modified by

successive researchers. In the AIT, engorged female ticks are treated with different concentrations of an acaricide in order to establish the discriminating dose (DD). This DD is the concentration required to kill 99% (LC_{99}) and 50% of the larvae (LC_{50}). This allows discriminating between susceptible and resistant ticks and calculating the resistance factors. The fecundity and fertility of the treated ticks is then compared with engorged female ticks which have not been treated with acaricides and resistance is then determined. Generally it may take up to 15 days before adult engorged females start laying eggs. A modified version of the AIT is the Larval Immersion test (LIT) which uses larvae instead of female adult ticks. The AIT is normally used as a preliminary screening test for acaricide resistance as it is faster than the larval packet test (LPT) and LIT, but less sensitive.

The larval packet test (LPT) uses acaricide-impregnated filter papers at different concentrations of the acaricide. The larvae are added into the filter papers and placed in an incubator (27-28°C temperature and 85-95% relative humidity) for 24 hours and the mortality is recorded for the different concentrations including the control. A dose-mortality curve is then plotted to compute the discriminating dose (DD). Initially engorged female adults are collected, they lay eggs which develop into larvae which are then used for the assay. It will therefore take approximately 4-8 weeks to have results of the LPT (Guerrero and Pruett, 2003). The LPT has been made a standard bioassay for the detection of acaricide resistance in ixodid ticks by the Food and Agricultural Organisation (FAO, 1984). The LPT has been found inaccurate in testing resistance for amitraz producing horizontal dose-response slopes (Kemp, 1998). This could be probably due to the short time ticks are exposed to the acaricide, the interaction between the chemical and the paper substrate and the instability of amitraz meaning it could be degraded during the assay. Miller et al. (2002) modified the LPT for amitraz resistance diagnosis by replacing filter papers with nylon fabric and using formulated amitraz containing stabilisers. This allowed determination of the DD.

A shortcoming of the LPT or any other bioassay is that there is little published information to guide predictions of field efficacy based on larval packet test. To at least identify resistance mechanisms with a LPT, synergists should be included as part of the

procedure (Foil et al, 2004). However these synergist are not specific in their mode of action. A more modified version of the LPT, the Larval Tarsal Test was recently developed (Lovis et al., 2013) where eggs are distributed in a microplates pre-treated with acaricides, this avoids the handling of larvae and will allowing testing a larger number of compounds and doses in a relatively short period of time as compared to the LPT.

2.6.5.2. Molecular Assays

The identification of resistance mechanisms in pyrethroids (He et al, 1999; Morgan et al., 2009, Jonsson et al., 2010a), organophosphates (Hernandez et al., 2000; Baffi et al., 2007) and amitraz (Chen et al., 2007; Baron et al, 2015) has enabled the development of molecular assays for the quick detection of acaricide resistance in *R. microplus*. Guerrero et al. (2001) developed an allele specific PCR based on the domain III mutation discovered by He et al. (1999) and this allowed detection of resistance in Mexican strains of *R. microplus*. Identification of additional mutations in the VGS domain II region (Morgan et al., 2009; Jonsson et al., 2010a) led to the development of a multiplex PCR by Lovis et al. (2012). The disadvantage with these allele specific PCR assays is that there is need to carry out separate reactions to identify susceptible and resistant mutations in one tick sample, making it a very long and tedious process. To solve this, a real-time based PCR was developed (Stone et al., 2014) which reduces testing time as there are no post PCR processes like gel-electrophoresis. In this study, a novel mutation in the domain II (super-kdr) which has been associated with extremely pyrethroid resistant insect species was identified in the *R. microplus* strain from Brazil. Studies employing the presence of mutations in the VGS have been carried out, Chen et al. (2009) using samples from Australia and Mexico confirmed the absence of the domain III mutation and the importance of this work was that they did not use the conventional allele-specific PCR but rather quantitative PCR methods based on melting temperature differences which allowed them to genotype the resistance status of the samples. This method proved to be much faster than the allele-specific PCR. A PCR-RFLP technique developed by Hernandez et al. (2002) could genotype pyrethroid resistance samples based on a point mutation in the carboxylesterase gene (Hernandez et al., 2000). This technique was also found useful in genotyping OP resistant *R.*

microplus strains from Brazil (Baffi et al., 2007) and was also adapted by Faza et al. (2013).

Baron et al. (2015) using mutations in the octopamine/tyramine receptor sequences identified by Chen et al. (2007) provided evidence that these SNPs were associated with amitraz resistance and developed a PCR-RFLP protocol to genotype resistance profiles of *R. microplus* populations to amitraz. The presence of a mutation associated with resistance will be very useful but the absence of a mutation should be interpreted with caution as this would not suggest the absence of resistance but rather alternative mechanisms of resistance (Jonsson and Hope, 2007). Work done by Miller et al. (1999), Li et al. (2004), Chen et al. (2009) support this statement.

The uptake of research in Africa on acaricide resistance has been very slow with few reports detailing acaricide resistance levels in some countries (Table 2-4). This was also observed by Jonsson and Hope (2007). Much work on acaricide resistance has been reported for the one-host tick *R. microplus* in Latin America and *R. australis* (previously *microplus*) in Australia. The absence of published reports on resistance does not translate to susceptibility of ixodid ticks to acaricides in use neither does it translate to resistance. It could be that studies are being carried out but they are not reaching the scientific domain or it could as well indicate the absence of research. In some cases, research has been carried out but resistance has not been detected (Adakal et al., 2013). It would be important to incentivise local researchers to publish their work for the greater benefit of the scientific community. The need to conduct more research in the area cannot be over emphasised. The resistance statuses of the acaricides being used in different countries need to be investigated and documented. Conventional bioassays still provide important information but there is a need to increase the usage of novel approaches like the molecular based assays. These will not only provide rapid information but might unravel as yet to be found mutations which will help in improving diagnostic assays or developing new acaricides.

Table 2-4: Published reports of acaricide resistance in African countries from 1980 to date (2017)

Acaricide class	Year resistance reported	Tick species	Country	References
Organophosphates (chlorfenvinphos, malathion, coumaphos)	1992 1987 2002	*R. decoloratus* *R. decoloratus* *R. decoloratus, R evertsi evertsi, A. hebraeum*[ER]	Zimbabwe Zambia South Africa	(Bruce and Mazhowu, 1992) (Luguru et al., 1987) (Mekonnen et al., 2002)
Pyrethroids (Cypermethrin, deltamethrin, flumethrin)	1992 2002 2016 2016	*R. decoloratus* *R. decoloratus* *R. microplus* *R. microplus*	Zimbabwe South Africa South Africa Benin	(Bruce and Mazhowu, 1992) (Mekonnen et al., 2002) (Robbertse et al., 2016)
Formamidines (amitraz)	2015 2016 2016	*R. microplus*[ER] *R. microplus* *R. microplus*	Zambia South Africa Benin	(Muyobela et al., 2015) (Baron et al., 2015)
Organochlorines (DDT, BHC and toxaphene)	1993 1991	*R. decoloratus* *R. decoloratus, R. microplus* *Rhipicephalus* and *Amblyomma spp.*	Ethiopia Tanzania	(Regassa and de Castro, 1993) (Kagaruki, 1991)

*ER- emerging resistance

2.7. Alternative tick control methods

In light of the emergence of resistance which is now a global problem. There is need to adopt alternative methods of tick control that will at least help in extending the useful life of existing acaricides (George et al., 2004). This is coupled also with the prohibitive costs associated with the development of a new acaricide product which has been conservatively estimated to exceed USD$100 million from discovery, development and global registration (Graf et al, 2004). Furthermore, the issues of public health and environmental safety have taken centre stage in as far as the continued use of acaricides is concerned (De Meneghi et al, 2016). These relate to acaricide residues in meat as well as milk which might have implications on human health due to the toxicity of these drugs and their persistence in the environment. Comprehensive reviews on alternative tick control methods which when applied in an integrated pest management programme will be sustainable have been reported (de Castro, 1997; George, 2000; Ghosh et al., 2007; Peter et al., 2005; Samish et al., 2004; Willadsen, 2006) and a summary will be given in this section.

One of the attractive control approach that has been proposed considering the serious drawbacks in acaricide use is the exploitation of the individual's immunity or tolerance especially in as far as the *Bos Indicus* tropical breeds which have co-evolved with tick parasites are concerned (Spickett et al., 1989). Apart from the ticks, the *Bos indicus* breeds have been shown to be more tolerant to tick-borne diseases as compared to the *Bos Taurus* breeds (Bock et al., 1997). Tick-host interactions are characterised by the development of acquired resistance which results in a reduction in the engorgement weight of the tick, number of viable ova produced, prevention of moulting and tick death (Singh and Girschick, 2003). The cattle immune response results in the production of antibodies which in the tick *R. microplus* has been found to work on the membrane glycol-proteins and the Bm86 glycoprotein. Willadsen et al. (1995) described the production of a recombinant vaccine (TickGARD^{TM}) from the Bm86 protein which results in a reduction in tick number on the tick by reducing the tick fertility and has a protective effect that lasts for six months. The draw back to this vaccine is that the efficacy varied between different tick strains hence the need for a polyvalent vaccine. An improved Bm86 vaccine Gavac^{TM} was developed in Cuba which offers partial protection to *Hyalomma* and *Rhipicephalus* tick species was found to be cost effective resulting in

savings of USD$23.40 per animal per year (de la Fuente et al., 1998). This vaccine could be useful particularly for the *Bos Taurus* cattle breeds which are more susceptible to ticks thus allowing the raising of these more productive breeds in tick endemic areas.

Vaccines are also available in the control of tick-borne diseases and their use has been prominent in Australia possibly impeding tick eradication efforts (George et al, 2002). Blood based vaccines are available for the control of bovine babesiosis infections (*B. bovis* and *B. bigemina*), anaplasmosis (*A. marginale*), hearwater (*Erlichia ruminantium*) while a stabilate vaccine exists for conferring protection against *Theileria parva* infections which cause Theilerosis (de Castro, 1997). Biological control of ticks although still in its infancy has also been proposed as part of an integrated pest management programme (Samish et al, 2004). This would involve the use of bacteria and entomopathogenic fungi such as *Metarhizium anisopliae* and *Beaveria bassiana* as well as entomopathogenic nematodes (Willadsen, 2006). Hendry and Rechav (1981) found out that bacteria would infect laboratory colonies of *Boophilus decoloratus*, *Amblyomma hebraeum*, *Hyalomma marginatum rufipes* and *Rhipicephalus evertsi evertsi* leading to blackening disease.

Other proposals which have been discussed include pasture management which could be a sustainable tick control method (Ghosh et al., 2007) but its more applicable in organised farming systems and will be a challenge to adopt in Zimbabwean communal farming systems which are characterised by sharing of grazing land. Pasture management involves activities such as pasture spelling, rotational grazing and cultivation of tick-trapping grasses such as Stylosanthes (George, 2000). Ethnoveterinary medicines are being adopted as natural acaricides (Ndhlovu and Masika, 2013; Nyahangare et al, 2015; Madzimure et al., 2013; Moyo and Masika 2009) and these are said to be cost-effective, environmentally friendly and offer low toxicity to animals, the challenge is that most of these have not been tested in the laboratories to determine the active ingredient.

Chapter 3: Shifts in the distribution of ixodid ticks parasitizing cattle in Zimbabwe

This chapter is based on:

1. Sungirai, M., Madder, M., Moyo, D.Z., De Clercq, P. & Abatih, E.N.(2015) An update on the ecological distribution of the Ixodidae ticks in Zimbabwe. *Experimental and Applied Acarology*, 66, 269–280.

2. Sungirai, M., Abatih, E.N., Moyo, D.Z., De Clercq, P. and Madder, M., 2017. Shifts in the distribution of ixodid ticks parasitizing cattle in Zimbabwe. *Medical and Veterinary Entomology*, *31*(1), pp.78-87.

3.1. Introduction

The distribution of ixodid ticks is not static, it is influenced by a number of factors such as animal movements, tick control strategies, resistance to acaricides and variations in rainfall (Tønnesen *et al*., 2004). Ixodid ticks parasitise a number of host animals but it is their presence on domestic animals that poses a threat to the livelihoods of people especially in sub-tropical areas (Jongejan & Uilenberg, 2004). Tick movement is facilitated by the movement of host animals from one area to another (Barré and Uilenberg, 2010). The continued presence of host animals together with suitable climatic conditions can lead to the establishment of a tick species in a given area (Lèger *et al*., 2013). Since ixodid ticks are important as vectors of causative agents of diseases of socio-economic importance in livestock, some of which are of zoonotic importance (Jongejan & Uilenberg, 2004), knowledge of tick distribution is relevant to understand risks of infection transmission and disease occurrence. The introduction of a tick species in an area has important implications on the epidemiology of the infections they transmit and subsequently on the livestock production potential of the area (Barré and Uilenberg, 2010).

A number of tick species are known vectors of disease causing pathogens as well as inflict direct damage on livestock in Africa (Horak *et al*., 2009; Spickett *et al*., 2011). Nationwide surveys on tick distribution in Zimbabwe were carried out between 1975-1980, 1988-1991, with the last known published survey conducted in 1996 (Peter *et al*., 1998a). This study aimed at providing an update on the distribution of ixodid ticks parasitising cattle in different ecological zones of Zimbabwe. This would help in assessing the potential shifts in the spatial occurrence of ixodid ticks parasitising cattle in Zimbabwe over the years. Such information will be crucial to animal health authorities for effective management and control of ticks and tick-borne diseases (TBDs) in this country (Bazarusanga et al., 2007).

3.2. Materials and Methods

3.2.1. Study area and sampling

In terms of agro-ecological areas, Zimbabwe is divided into five regions (Gambiza and Nyama, 2000). The ecological zones are shown on Figure 3-1 and their characteristics summarised in . Ecological regions 1, 2 and 3 are also referred to as the Highveld, while regions 4 and 5 are referred to as the Lowveld (Norval et al., 1994).

Table 3-1: Characteristics of the ecological regions of Zimbabwe

Ecological region	Characteristics of region
1	>1000mm rainfall; mean annual temperature range of 15-18 ºC, mean minimum temperature range of 10-12 ºC and mean maximum temperature range of 19-23ºC; tea, coffee, plantation farming, macadamia, fruits, intensive livestock production.
2	750-100mm rainfall; mean annual temperature range of 16-19 ºC , mean minimum temperature range of 10-13 ºC and mean maximum temperature range of 19-23 ºC; intensive crop and livestock production.
3	650-800mm of rainfall; mean annual temperature range of 18- 22 ºC; mean minimum temperature range of 11-15 ºC and mean maximum temperature range of 23-26 ºC. severe mid-summer droughts but maize, tobacco, cotton and other cash crops grown
4	650- 800mm of rainfall; ean annual temperature range of 18-24 ºC, mean minimum temperature range of 11-20 ºC and mean maximum temperature range of 19-26 ºC; livestock and drought resistant crop production
5	<450mm rainfall; mean annual temperature range of 21-25 ºC, mean minimum temperature range of 14-18 ºC and mean maximum temperature range of 26-32 ºC; supports extensive cattle or game protection

Multi-stage sampling was done where sampling was done for the districts within a province, dipping tanks within a district and cattle (primary sampling unit) within a dip tank. Following a literature search (from the internet search engines such as Google, Google Scholar and records at the Department of Veterinary Services archives) no reliable estimate of the dip tank prevalence for each tick species could be obtained so an estimate of 50% was assumed as suggested by Thrusfield (2005). Therefore, the total number of dip tanks to be selected throughout the country was calculated to be 384, with a 95% Confidence Interval (CI). This translated to approximately 77 dip tanks per ecological zone on average, although this varied depending on the size of the ecological zone. The technical and logistical support for this study relied much on the network and personnel of the Department of Veterinary Services which is administered through provinces and districts. The country is made up of 59 districts within 11 provinces. Sampling was to be done in 55 districts and 7 dip tanks per district. However, accessibility to some areas was impossible due to either terrain or resource limitations and farmers' willingness to participate in the survey, hence not all dip tanks and districts were sampled.

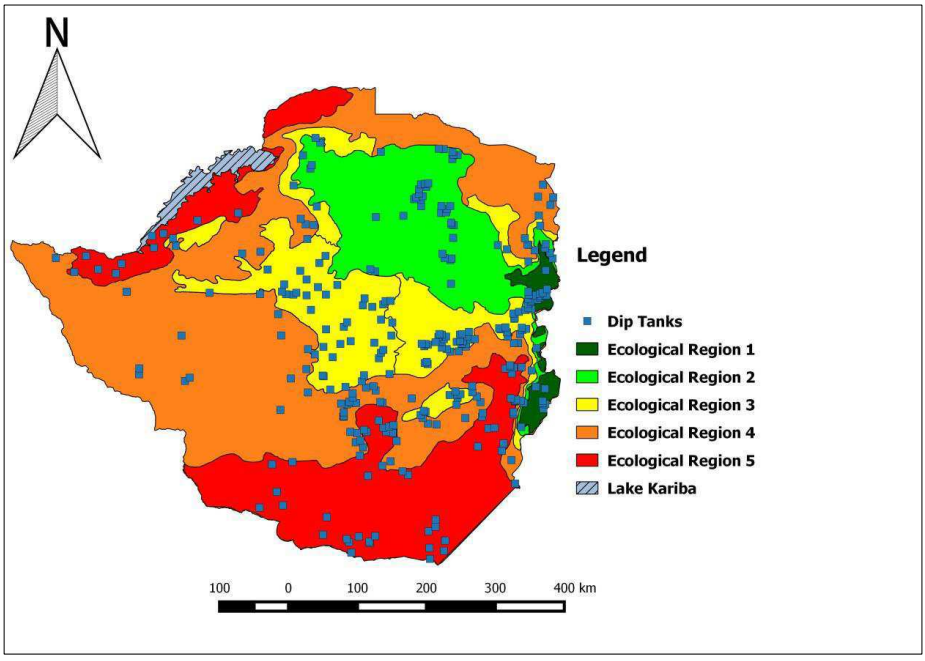


Figure 3-1: Map showing the ecological regions of Zimbabwe and the dip tanks at which tick collections were performed on cattle

A sampling frame of the total number of districts in the province and dip tanks in the district was obtained from the local veterinary office. Random selection of the districts and dip tanks was done by assigning a number to each element (district / dip tank). Random numbers, as many as the number of districts and dip tanks, were generated using Microsoft Excel 2007. The districts were then sampled according to the order of the assigned random numbers. Within each district, 7 dip tanks were sampled according to the first (i.e. 1-7) numbers randomly assigned. Ixodid ticks were collected from a total of 322 dip tanks (Figure 3-1) in 39 districts within 9 provinces. The metropolitan provinces of Harare and Bulawayo were excluded since they comprised urban settlements where communal cattle farming is not practised. Ticks were collected between September 2013 and May 2015. The collections were performed on at least 5 heavily infested cattle per dip tank (Horak *et al*., 2009; Norval *et al*., 1984). The cattle were considered heavily infested according to the Animal Health Act (Cattle Cleansing) Regulation from 1993, which recognizes the presence of 10 or more live ticks on the animal or 5 or more engorged ticks present on each of 5 animals or more in a herd

(Ndhlovu *et al*., 2009). Using steel forceps, a sub-sample of the ticks present on cattle was collected from all the predilection attachment sites which were: the base of tail, perianal region, perineum, legs, axillae, hooves, udder, scrotum, belly, dewlap, head and ears (De Clercq *et al*., 2012). Adult tick specimens were collected to allow morphological identification up to the species level (Horak et al., 2009; Lorusso et al., 2013; Nyangiwe et al., 2013a). The total number of ticks collected from each cattle and at each dip tank was recorded. The morphological identification of ticks was done using identification keys as provided by Walker *et al*. (2003) as well as those by Walker *et al*. (2000) for the *Rhipicephalus* species.

3.2.2. Data analysis

Descriptive statistics were used to analyse the data by calculating the dip tank prevalence (i.e. ratio of dip tanks where a particular species were found to the total number of dip tanks sampled) and mean infestation rate per cattle, together with the corresponding 95% CI and standard errors of the mean respectively. Comparisons between the prevalence were done based on the 95% CI with overlapping intervals suggesting no significant differences between the prevalence of two tick species. Maps to show the distribution of each tick species were constructed using the Quantum GIS software (QGIS Development Team, 2013).

3.3. Results

3.3.1. Dip tank prevalence of ixodid tick species

A total of 21 954 adult hard ticks were collected from 1 355 cattle during the survey. The dip tank prevalence together with the confidence intervals are presented in Table 3-1. Tick species identified included *Amblyomma hebraeum* (65.2%, n=210/322), *Rhipicephalus evertsi evertsi* (65.2%, n=210/322), *Hyalomma rufipes* (62.4%, n=201/322), *Rhipicephalus appendiculatus* (60.6%, n=195/322), *Rhipicephalus decoloratus* (61.8%, n=199/322), *Hyalomma truncatum* (37.9%, n=122/322), *Rhipicephalus microplus* (32%, n=103/322), *Amblyomma variegatum* (14.9%, n=48/322). The brown ticks *Rhipicephalus* near *punctatus* (7.1%, n=23/322), *Rhipicephalus simus* (5.6%, n=18/322), *Rhipicephalus lunulatus* (4%, n=13/322), *Rhipicephalus* cf. *turanicus* (3.4%, n=11/322) and *Rhipicephalus compositus* (0.3%,

n=1/322) were less common. Ticks of the *R. turanicus* species were identified as *R.* cf. *turanicus* because of their morphological differences (i.e. denser punctations on the *scutum* and more narrow angular adanal plates), enabling to distinguish Southern African specimens from those from North Africa, the Middle East and the Far East (Beati and Keirans, 2001).

3.3.2. Ecological distribution of ixodid tick species

Distribution of collected ticks according to the ecological zone is illustrated in Figure 3-2 and Figure 3-3. Prevalence of each tick species according to the ecological zone is presented in Table 3-1. *Rhipicephalus decoloratus* and *R. evertsi evertsi* were the most common tick species in all the ecological regions. *Amblyomma variegatum*, *H. rufipes* and *R. decoloratus* were seen to be more widespread in the arid Zambezi valley where there were also pockets of occurrences of *R. microplus* (see Figure 3-3). *Rhipicephalus microplus* was more prevalent in ecological regions 1 and 2. *Amblyomma variegatum* was not found in ecological region 1 whilst *A. hebraeum* had a patchy distribution in the region, the same observation was made for the tick species *H. rufipes* and *H. truncatum*. The lesser known *Rhipicephalus* species (*R.* near *punctatus*, *R. simus*, *R. lunulatus*, *R.* cf. *turanicus* and *R. compositus*) had a sparse distribution, being virtually absent in most areas.

Table 3-2: Mean tick burden on cattle (n=1355) and at various dip tanks and associated prevalences (cattle, diptank and ecological region)

Tick species	Total number of ticks collected	Mean tick burden on cattle +/- standard error	Prevalence of ticks on cattle /%	95% Confidence Interval of prevalence on cattle		Dip tank prevalence of ticks / % (n=322)	95% Confidence Intervals for dip tank prevalence		Ecological Region Prevalence /% with 95% Confidence Interval Estimates				
				Lower limit	Upper limit		Lower limit	Upper limit	1(n=15 dip tanks)	2 (n=62 dip tanks)	3 (n=107 dip tanks)	4 (n=71 dip tanks)	5 (n=67 dip tanks)
Amblyomma hebraeum	5151	7.5+/-0.3	50.9 (n=690)	48.2	53.6	65.2 (n=210)	60.0	70.4	2.7 (0-10.9, n=4)	21 (10.9-31.1, n=13)	86 (79.4-92.6, n=92)	76 (66.1-85.9, n=54)	70.1 (59.1-81.1, n=47)
Amblyomma variegatum	776	7.2+/-0.9	8 (n=109)	6.6	9.4	14.9 (n=48)	11.0	18.8	0(n=0)	30.6 (19.1-42.1, n=19)	3.7 (0.1-7.3, n=4)	7 (1.1-12.9, n=5)	14.9 (6.4-23.4, n=10)
Hyalomma rufipes	2317	4.8+/-0.3	35.4 (n=480)	32.9	37.9	62.4 (n=201)	57.1	67.7	40 (15.2-64.8, n=6)	46.8 (34.4-59.2, n=29)	66.4 (57.5-75.3, n=71)	59.2 (47.8-70.6, n=42)	79.1 (69.4-88.8, n=53)
Hyalomma truncatum	796	3.7+/-0.3	15.7 (n=213)	13.8	17.6	37.9 (n=122)	32.6	43.2	26.7 (4.3-49.1, n=4)	59.7 (47.5-71.9, n=37)	35.5 (26.4-44.6, n=38)	28.2 (17.7-38.7, n=20)	34.3 (22.9-45.7, n=23)
Rhipicephalus appendiculatus	2448	5.9+/-0.3	30.8 (n=418)	28.3	33.3	60.6 (n=195)	55.2	65.9	73.3 (50.9-95.7, n=11)	85.4 (76.6-94.2, n=53)	68.2 (59.4-77, n=73)	46.5 (34.9-58.1, n=33)	37.3 (25.7-48.9, n=25)
Rhipicephalus compositus	3	3+/-0	0.07 (n=1)	-0.1	0.2	0.3 (n=1)	0	0.9	0(n=0)	1.6 (0-4.7, n=1)	0(n=0)	0(n=0)	0(n=0)
Rhipicephalus decoloratus	5239	9.4+/-0.5	40.1 (n=555)	37.5	42.7	61.8 (n=199)	56.5	67.1	53.3 (28.1-78.5, n=8)	94.5 (88.8-100, n=58)	70.1 (61.4-78.8, n=75)	74.6 (64.5-84.7, n=53)	37.3 (25.7-48.9, n=25)
Rhipicephalus evertsi evertsi	2379	4.5+/-0.3	39.3 (n=532)	36.7	41.9	65.2 (n=210)	60.0	70.4	73.3 (50.9-95.7, n=11)	45.2 (32.8-57.6, n=28)	84.1 (77.2-91, n=90)	54.9 (43.3-66.5, n=39)	62.7 (51.1-74.3, n=42)
Rhipicephalus lunulatus	79	4.2+/-0.7	1.4 (n=19)	0.8	2.0	4.0 (n=13)	1.9	6.2	0(n=0)	0(n=0)	10.3 (4.5-16.1, n=11)	2.8 (0-6.6, n=2)	25.4 (0-0(n=0)
Rhipicephalus microplus	2307	8.5+/-0.5	20 (n=271)	17.9	22.1	32.0 (n=103)	26.9	37.1	73.3 (50.9-95.7, n=11)	46.8 (34.4-59.2, n=29)	28 (19.5-36.5, n=30)	22.5 (12.8-32.2, n=16)	25.4 (15-35.8, n=17)
Rhipicephalus near *punctatus*	180	5.5+/-0.9	2.4 (n=33)	1.6	3.2	7.1 (n=23)	4.3	10.0	33.3 (9.5-57.2, n=5)	11.3 (3.4-19.2, n=7)	5.6 (1.2-10, n=6)	5.6 (0.3-11, n=4)	1.5 (0-4.4, n=1)
Rhipicephalus simus	95	4.1+/-0.9	1.7 (n=23)	1.0	2.4	5.6 (n=18)	3.1	8.1	6.7 (0-19.4, n=1)	4.8 (0-10.1, n=3)	9.3 (3.8-14.8, n=10)	2.8 (0-6.6, n=2)	3.0 (0-7.1, n=2)
Rhipicephalus turanicus	119	9.2+/-3.5	1.0 (n=13)	0.5	1.5	3.4 (n=11)	1.4	5.4	0(n=0)	6.5 (0.4-12.6, n=4)	4.7 (0.7-8.7, n=5)	4 (0-8.6, n=1)	1.5 (0-4.4, n=1)

3.3.3. Prevalence of ticks on cattle

The prevalence of the tick species on cattle are presented in Table 3-1: Mean tick burden on cattle (n=1355) and at various dip tanks and associated prevalences (cattle, diptank and ecological region), together with the confidence intervals. *Amblyomma hebraeum* (n=5151) was the most common tick species on cattle being recorded on 690 animals followed by *R. decoloratus* (n=5239, 555 cattle) and *R. evertsi evertsi* (n=2379, 532 cattle). In descending order, the following tick species were commonly found on cattle, namely: *H rufipes* (n=2317, 480 cattle), *R. appendiculatus* (n=2448, 418 cattle), *R. microplus* (n=2307, 271 cattle), *H. truncatum* (n=796, 213 cattle), *A. variegatum* (n=776, 109 cattle), *R.* (near) *punctatus* (n=180, 33 cattle), *R. simus* (n=95, 23 cattle), *R. lunulatus* (n=79, 19 cattle), *R.* cf. *turanicus* (n=119, 13 cattle) and *R. compositus* (n=3, 1 cattle).

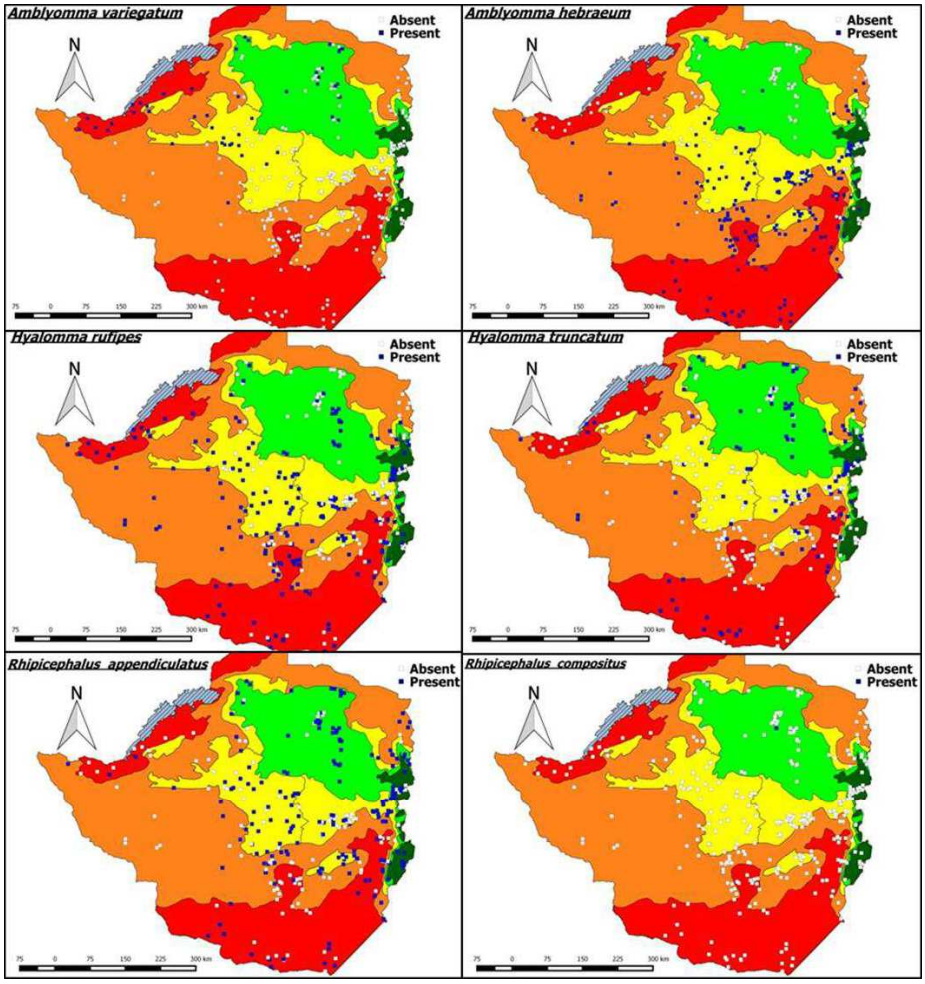


Figure 3-2: Distribution of ixodid ticks in Zimbabwe: *Amblyomma variegatum, Amblyomma hebraeum, Hyalomma rufipes, Hyalomma truncatum, Rhipicephalus appendiculatus and Rhipicephalus compositus*.

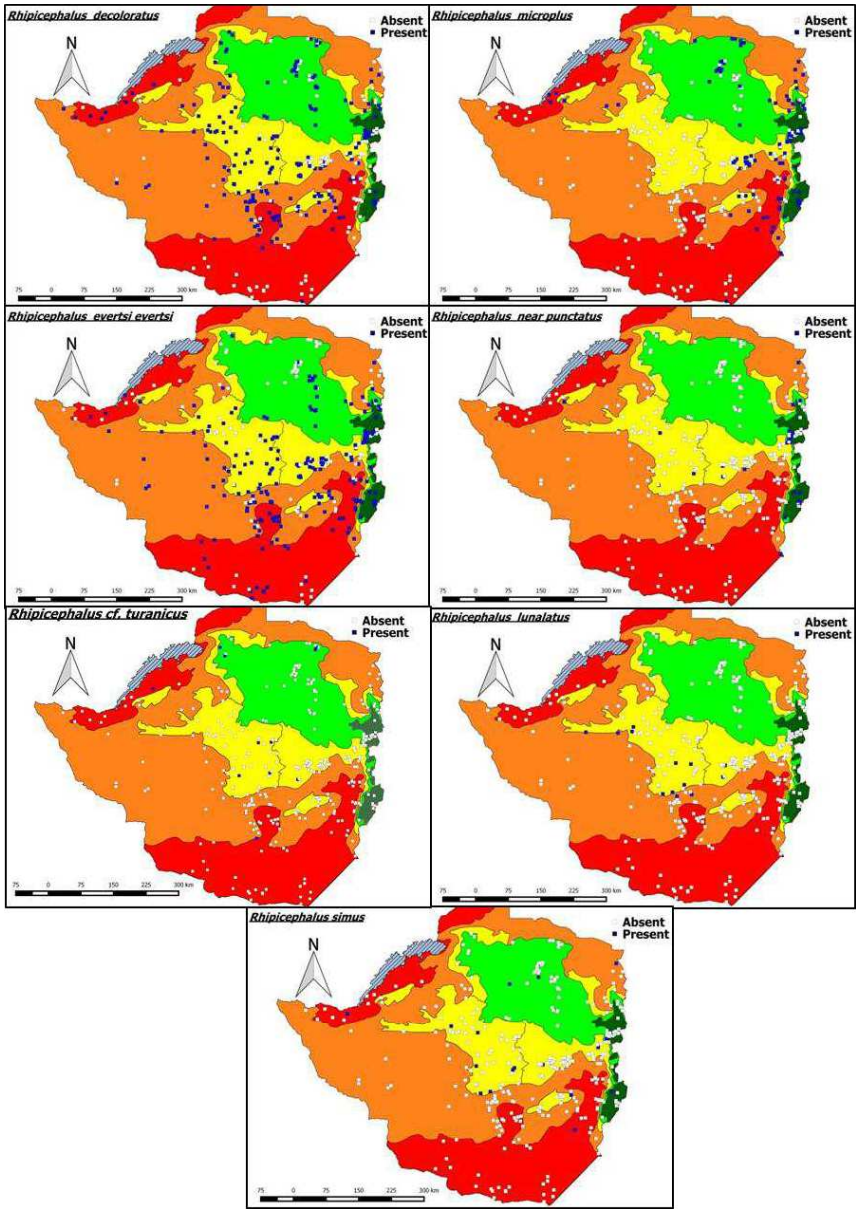


Figure 3-3: Distribution of ixodid ticks in Zimbabwe: *Rhipicephalus microplus, Rhipicephalus decoloratus, Rhipicephalus evertsi evertsi, Rhipicephalus near punctatus, Rhipicephalus turanicus, Rhipicephalus lunalatus and Rhipicephalus simus.*

3.4. Discussion

Nationwide surveys on the distribution of ixodid ticks in Zimbabwe were previously carried out between 1975-1980, 1988-1991 and in 1996 (Peter et al., 1998a). In this study, it was observed that whilst the distribution of other ixodid tick species has remained unchanged, there have been changes in the distribution of *A. variegatum*, *A. hebraeum* and *R. microplus*. Although the sampling strategy was designed in a standardized way with a view of getting as much a representative sample as possible, this could not be entirely achieved. In some cases, sampling was influenced by the availability of resources, accessibility of dip tanks and farmers willingness to participate in the survey. Such limitations were also experienced by Peter et al. (1998a) and De Clercq et al. (2012).

Amblyomma variegatum and *A. hebraeum* are normally parapatric species. In the Sub-Saharan region, *A. variegatum* has a wide distribution with a southern limit in Mozambique, Zimbabwe and Botswana while a northern limit is observed for *A. hebraeum* which is also present in South Africa and Swaziland (Bournez et al., 2015). In Zimbabwe, the traditional foci of *A. variegatum* have been the west and north-western parts of Zimbabwe corresponding to the Lowveld region. The results of this survey indicated that this tick species has moved northwards, being common in ecological region 2 of the country which is in the north-eastern Highveld. The tick species continues to be abundant in the Zambezi Valley which is in the northern Lowveld region. It is also important to note that *A. variegatum* was not collected in the eastern Highveld and western Lowveld parts of the country in sharp contrast to past reports (Peter et al., 1998a). However, *A. hebraeum* is still not common in the northern Highveld where *A. variegatum* has now shifted to and is the dominant species. The climatic niches of these two *Amblyomma* species are different (Estrada-Peña et al., 2008), but the most important factor influencing their distribution is the range of available alternative hosts, especially wildlife (Norval et al., 1994). In Zimbabwe, *A. hebraeum* infests a wide range of wildlife species, whilst the wildlife host range of *A. variegatum* seems to be limited to the buffalo (*Syncerus caffer*) (Norval et al., 1994). Moreover, in Zimbabwe, the distribution of the buffalo has been confined to the National Parks to avoid spread of diseases like Foot and Mouth (FMD) while other wildlife species such as the giraffe (*Giraffa camelopardalis*), kudu (*Tragelaphus strepsiceros*), eland (*Taurotragus oryx*) and

warthog (*Phacochoerus africanus*), which can be alternative hosts for *A. habreum,* are widespread in the country (Norval et al., 1994). This could explain the expansion of the distribution of *A. hebraeum* in the western Lowveld where there is the Hwange National Park, the habitat of several ungulate species which can serve as hosts for the ticks.

In addition, in the eastern highlands (Highveld) there has been a noticeable increase in commercial wildlife farming which would provide alternative hosts for *A. hebraeum*. Furthermore, according to previous studies (Estrada-Peña et al., 2008), the expansion of *A. hebraeum* in the Highveld could be driven by intense periods of drought. Accordingly, in the north-eastern Highveld, *A. variegatum* has a high prevalence which could be attributed to the warmer temperatures and less intense dry periods observed in this area (Estrada-Peña et al., 2008). In the present study the co-existence of the *Amblyomma* species was observed in the central Highveld and this corroborates previous observations (Peter et al., 1998a). When these two species have overlapping distributions, *A. variegatum* was observed to dominate (Norval, 1983). The central area would serve as a hybrid zone limiting the spread of either of the tick species down south or up north (Sutherst, 1987). In this zone, there is exclusive competition between the two species which results in a parapatric distribution (Norval et al., 1994; Rechav et al., 1982). The parapatric relationship between *A. hebraeum* and *A. variegatum* could be further explained by the occurrence of competition between these two species for the same attachment sites on the host, leading to reproductive interference and cross-mating (Bournez *et al*., 2015).

Rhipicephalus microplus was found to be present in the interior region (south-eastern Lowveld and northern Highveld) of the country as well as in the northern Lowveld with appreciable occurrences in areas close to Lake Kariba. There have been no records of the occurrence of this tick species in this area either in the published literature (Katsande et al., 1996; Norval et al., 1983) or according to the authors' knowledge. The traditionally known areas for *R. microplus* have been the eastern Highveld which is characterized by warmer temperatures and higher rainfall (Katsande et al., 1996). Although this tick species has spread to other areas, it is seen to be largely confined to the Highveld region. These areas have warmer temperatures and high rainfall (Gambiza & Nyama, 2000), creating suitable habitats for *R. microplus*. It was also interesting to note the presence of *R. microplus* both in the southern and northern Lowveld of the

country, although these areas do not possess a suitable climate for the proliferation of this tick species. This could be attributed to movement of animals, especially cattle, which increased in particular during and after the land reform programme in Zimbabwe (Mavedzenge et al., 2006).

In contrast, *R. decoloratus* has a wider distribution than *R. microplus*. This could be attributed to the fact that *R. decoloratus* tolerates a wide range of temperature and rainfall conditions and has the ability to infest alternative hosts including wildlife species (Lynen et al., 2008). The complete displacement of *R. decoloratus* by *R. microplus* which has been recorded in other countries (De Clercq et al., 2012; Nyangiwe et al., 2013a; Tønnesen et al., 2004) has so far not been apparent in Zimbabwe. In this study, indeed, *R. decoloratus* occurred in approximately 50% (52/103) of the dip tanks where *R. microplus* was recorded. The reasons for this current balanced co-occurrence could be related to the presence of alternative hosts for *R. decoloratus* despite the relative reproductive advantage of *R. microplus*. The presence of alternative hosts particularly in colder and dry areas will tend to reduce the competitive advantage that *R. microplus* has over *R. decoloratus* (Sutherst, 1987). Another factor that could lead to the failure of *R. microplus* to displace *R. decoloratus* is the tick control strategy being adopted in Zimbabwe. Over the years, the country has embarked on government subsidised dipping where tick control in the communal areas which have more than 60% of the cattle population is done weekly during the rainy season and fortnightly in the dry season (Peter et al., 1998b). This kind of tick control is intensive and it has been observed that more than 70% of communal farmers participate in these programs (Chapter 6). Although the resistance status of these two species is not known in Zimbabwe, it has been reported that *R. decoloratus* would be more resistant to acaricides as compared to *R. microplus* (Baker et al., 1981) and this would ensure a gradual displacement of the former (Lynen et al., 2008).

Other tick species collected in this study were *H. rufipes*, *H. truncatum*, *R. appendiculatus*, *R. compositus*, *R. evertsi evertsi*, *R. lunulatus*, *R.* near *punctatus*, *R. simus* and *R.* cf. *turanicus*. *Hyalomma* species tolerate a wide range of climatic environments although they are common in the most arid regions of the tropics (Walker et al., 2003). The distribution of *H. truncatum* is expected to be wider than that of *H. rufipes*, this is because the former parasitises a diverse number of hosts while the latter

prefers larger wild ungulates at the adult stage (Norval, 1982). Since this study was focused on sampling from cattle, this could explain the wider distribution and higher prevalence of *H. rufipes* as compared to *H. truncatum*. In this study, *R. appendiculatus* was one of the most common tick species collected and had a higher prevalence in the Highveld region compared to the Lowveld. The climatic conditions of high rainfall, cooler temperatures and host availability (Hove et al., 2008) provide a suitable environment for the proliferation of *R. appendiculatus* in this region. *Rhipicephalus evertsi evertsi* is regarded as the most widely distributed *Rhipicephalus* species in sub-Saharan Africa (Horak et al., 2009). In this study, it had the highest prevalence (65.2%) at the dip tank level together with *A. hebraeum* and was also widely distributed in all the ecological regions of the country.

In the present study *R.* (near) *punctatus* had a patchy distribution in all the ecological regions of the country although it was most common in the Highveld. In sub-Saharan Africa, *Rhipicephalus* (near) *punctatus* is found mainly in Zimbabwe, Zambia and Northern Mozambique (Guglielmone et al., 2014) being present in tropical and sub-tropical grasslands as well as savannas and shrub lands. *Rhipicephalus simus* is a widely distributed tick species in Zimbabwe (Walker et al., 2003), although there were very few collections in this study. As noted by Peter et al., (1998a), such kind of studies on the occurrence of ixodid ticks in cattle although very sensitive have a high risk of yielding false negatives. This applies to all other tick species in this study and those that were not observed at all, more so for *R. simus* which has a predilection for the tail brush and around the feet of cattle. These attachment sites are normally overlooked during tick collection, an observation also noted by Spickett et al., (2011). *Rhipicephalus lunulatus* was only found in ecological regions 3 (Highveld, semi -intensive farming) and 4 (Lowveld, semi-extensive farming). In ecological region 4, *R. lunulatus* was found in areas adjacent to ecological region 3 confirming reports of Walker et al., (2003) that this tick species is widespread in Savanna climates.

There were isolated occurrences of *R.* cf. *turanicus* in this study and this conforms with reports of Walker et al., (2003). There was one collection of *R. compositus* in the Highveld region, this tick species is expected to be common in this region at medium to high altitudes and mean annual rainfall of above 700 mm (Walker et al., 2000). The low prevalence of *R. compositus* might be related to false negative results associated with

such types of studies (Peter et al., 1998a). In addition, the immature stages of this tick are common on creek rats (*Pelomys fallax*) and these may contribute to the abundance of adult individuals of this tick (Walker et al., 2000). Although the distribution of *P. fallax* was not investigated in this study and is not known in Zimbabwe, the collection of *R. compositus* has been associated with areas where the creek rats have been recorded (Walker et al., 2000).

In the light of the consistent dipping practices by communal farmers in Zimbabwe reported in Chapter 6, the mean tick burden recorded on cattle in this study was considered as relatively high (Lorusso et al., 2013) for most species. This could be attributed to the likely emergence of acaricide resistance especially to amitraz which is the one commonly used by farmers to control ticks. This is especially seen for the one host ticks, *R. decoloratus* and *R. microplus,* which recorded the highest tick burdens as compared to other tick species. One host ticks are known to develop resistance more readily than two or three host tick species (Mekonnen et al., 2002). Future studies would be desirable to assess the status of acaricide resistance in boophilid ticks of Zimbabwe.

The widespread distribution of *A. hebraeum*, *R. evertsi evertsi*, *R. decoloratus* and *R. appendiculatus* might have implications in cattle producing areas on the epidemiology of heartwater, anaplasmosis, babesiosis and theileriosis, respectively. Furthermore, the spread of *A. variegatum* may have serious implications on the occurrence of dermatophilosis as was noted during field observations in this study. In indigenous cattle producing areas, the diseases anaplasmosis and babesiosis are usually characterized by endemically stable situations when tick control is minimal (Norval et al., 1983, 1984). Short interval dipping may disrupt endemic stability and increase susceptibility of cattle to the diseases and when the supply of acaricides becomes inconsistent this may lead to cattle mortalities as it has been observed in the past (Norval et al., 1983, 1984). The situation might be different for heartwater and theileriosis (by *Theileria parva*) where endemically stable situations are rare (Irvin et al., 1996; Minjauw and Mcleod, 2003).

In conclusion, tick diversity and abundance is relatively high on cattle. This will negatively affect productivity through direct losses notwithstanding the indirect losses through pathogen transmission. It may be necessary to describe other areas which maybe suitable for occupation by the ticks which are of veterinary importance. This is

crucial for risk analysis and surveillance purposes. The next chapter will focus on describing habitat suitability of *Rhipicephalus microplus* a highly invasive tick parasite whose geographic range was seen to be expanding. This will be done together with the indigenous competitor *R. decoloratus* to explore the different requirements for the two tick species and find out if there is overlap.

Chapter 4: Modelling the distribution of *Rhipicephalus microplus* and *Rhipicephalus decoloratus* in Zimbabwe

4.1. Introduction

The invasive tick species *Rhipicephalus microplus* is regarded as the most important cattle tick in the world (Giles et al., 2014). Having originated from South East Asia, *R. microplus* has spread globally, and is now found in most tropical and subtropical countries (Estrada-Peña et al., 2006a). The invasiveness of this tick species has been largely attributed to its high reproductive capacity characterised by a short life cycle, its high adaptability to changing environments, its increasing resistance to acaricides and the fact that it is a one host tick benefitting from cattle movement for spreading efficiently (Barré and Uilenberg, 2010). The introduction of this species in an area becomes a concern to disease control authorities as not only does it cause production losses in cattle but it transmits the more pathogenic form of bovine babesiosis (*Babesia bovis*) which may be a threat to indigenous cattle. Even more, the opportunities for keeping the more superior exotic or cross breeds in these areas is jeopardised as these are more susceptible to *B. bovis* infections.

Control strategies for invasive vector species involve identifying risk areas within which these species can potentially establish upon introduction (Estrada-Peña, 1999). Areas deemed suitable for the tick species but not yet invaded may be put under surveillance with constant inspection of cattle moving into these areas for the presence of ticks. Eradication programmes may be organised if the tick species is located in isolated areas with a potential to spread. These areas provide the right environmental conditions for the survival and proliferation of the species. For tick species, these are climate, vegetation and availability of suitable hosts (De Clercq et al., 2013; Estrada-Peña, 2008). During the parasitic phase, the host provides all resources required for tick development, but during the pre- and post-parasitic phases, favourable climatic conditions and vegetation patterns are essential. It has been proposed that of these factors, climate is the most limiting factor for tick distribution (Cumming, 2002).
A common approach in modelling species distribution is the use of a generalised linear model (GLM) for presence and absence data using environmental variables as predictors of species occurrence (Hijmans and Elith, 2016). Data on weather and

climate are routinely obtained from weather stations around the world or from satellite imagery. For climate data, the WorldClim dataset is often used and sensors like the Moderate Resolution Imaging Spectroradiometer (MODIS) are useful in gathering environmental data (De Clercq et al., 2015). The final output of species modelling is a suitability map which shows a degree of suitability for each pixel, where values close to '1' indicate the areas that are highly suitable and values close to '0' indicate the areas that are unsuitable for the occurrence of a species.

In Chapter 3 the results of the nationwide tick survey suggested an expansion in the geographic range of *R. microplus* and a partial displacement of the more drought-tolerant *Rhipicephalus decoloratus* by the former. This is despite previous reports of complete displacement of *R. decoloratus* by *R. microplus* in the eastern Zimbabwe (Mason and Norval, 1980). Norval et al., (1992a) reported that because of the 1981-1984 drought experienced in Zimbabwe, *R. microplus* could have completely disappeared. Later studies however showed that the tick was present in the east and north east parts of the country (Katsande et al., 1996). In other countries *R. microplus* has been reported to displace *R. decoloratus* (Berkvens et al., 1998; De Clercq et al., 2012; Nyangiwe et al., 2013a; Tønnesen et al., 2004).

The expansion in the range of *R. microplus* and the possible displacement of *R. decoloratus* raise two questions: (i) Are there other areas in Zimbabwe where climatic conditions are suitable for *R. microplus* to exist, survive and reproduce and is not presently reported? and (ii) do the two tick species *R. microplus* and *R. decoloratus* share the same ecological niche in Zimbabwe? This study seeks to model the habitat suitability of these competing tick species, the autochthonous *R. decoloratus* and the invasive *R. microplus,* and to determine whether their niche overlaps. Previous papers have predicted the habitat suitability of these two tick species on a continental scale using tick records from 1900 to 1990 (Estrada-Peña et al., 2006a). However, as more recent tick occurrence data became available, it may be necessary to update these species distribution maps (Hahn et al., 2016). This will provide updated information to the livestock production sector, allowing animal health authorities to evaluate the control programmes in place.

4.2. Methodology

4.2.1. Study area

This study used data of *R. microplus* and *R. decoloratus* ticks collected in Zimbabwe (Figure 4-1) during a survey conducted on cattle at communal dipping tanks from September 2013 to May 2015. The details on sample collection are described in detail in Chapter 3. *Rhipicephalus microplus* ticks were found at 32% (103/322) of the dipping tanks while *R. decoloratus* ticks were found at 62% (200/322) of the dipping tanks. Figure 4-1 shows the location of the dipping tanks where cattle were screened for ticks and the respective occurences of the two tick species.

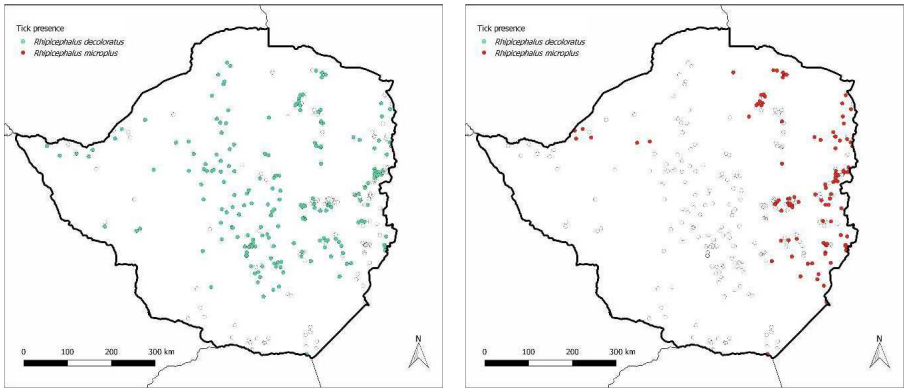


Figure 4-1: Data on tick occurrence of *Rhipicephalus decoloratus* (left) and *Rhipicephalus microplus* (right) in Zimbabwe

4.2.2. Environmental variables

The predictor variables used in the study were the 19 bioclimatic variables (Table 4-1) obtained from the WorldClim dataset which is an interpolated dataset of temperature and precipitation data obtained from weather stations for the period 1950-1990 (Hijmans et al., 2005). Other predictor variables were the Normalised Difference Vegetation Index (NDVI) for the period January 2013- December 2015 obtained by remote sensing from the MODIS sensor (Justice et al., 1998). Monthly NDVI values (MOD13A3) were averaged into mean, maximum and minimum NDVI values for each dip tank. The NDVI is a measure of photosynthetic activity which reflects available moisture on the ground (Randolph, 2010), its values range from – 1 to +1, with negative values indicating snow

while values close to zero indicate bare soil and values closer to one indicate a lot of greenness.

4.2.3. Model building

The procedure described by Cumming (2002) was used to build the models. The presence-absence data for both tick species was divided into a training set (75%) for model calibration and a test set (25%) for model evaluation. First, univariate logistic regression was done using a generalised linear model (GLM) with the "binomial logit" link function to model the association between each variable and tick occurrence. Variables which were significantly correlated (p-value < 0.05) with tick presence were retained as potential predictors for habitat suitability (see Table 1). With these, multivariate analyses were done using the stepwise method for model selection (Hirzel et al., 2006), where the model with the lowest Akaike Information Criterion (AIC) was selected. The model chosen as the best was run and the significant variables ($p<0.05$) were concluded to be the best predictors for habitat suitability.

4.2.4. Model Evaluation

The test set was used to evaluate the predictive power of the model. The Area Under the ROC-Curve (AUC) was used since it will be independent of the prevalence in this case of the tick species (Pearce and Ferrier, 2000). Another important feature of the AUC is that it is not affected by collinearity and spatiotemporal autocorrelation (Cumming, 2002). AUC values of 0.5-0.7 indicate low accuracy, 0.7-0.9 useful applications and > 0.9 high accuracy (Manel et al., 2001). Afterwards, the correlation between the two resulting suitability maps was assessed using the Kendall rank correlation coefficient, a non-parametric test for dependence.

4.2.5. Environmental requirements for the two tick species

Probability density distributions and bivariate plots were computed to show the range of environmental conditions where the two tick species occurred and compare the requirements for the two tick species.

4.2.6. Software used

All statistical analyses were carried out in the R statistical package version 3.2.3 (R Development Core Team, 2013). The following packages in R were used, “raster”, “rgdal”, “dismo”, “maptools”, “sp”, “RODBC”, “caret”, “mlbench” and “MASS”. All maps were prepared using QGIS software (QGIS Development Team, 2013).

4.3. Results

4.3.1. Univariate analysis

Of the 22 variables, 16 were found to be significantly correlated to the presence of *R. microplus* prediction. These were: altitude, annual mean diurnal range, isothermality, temperature seasonality, maximum temperature of warmest month, minimum temperature of coldest month, annual temperature range, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation of wettest quarter, precipitation of driest quarter, precipitation of coldest quarter, precipitation of warmest quarter, mean monthly NDVI and minimum NDVI values. Fourteen variables were found to be significant predictors ($p<0.05$) for *R. decoloratus* and these were: altitude, annual mean temperature, isothermality, temperature seasonality, maximum temperature of warmest month, minimum temperature of coldest month, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest month, precipitation seasonality and precipitation of wettest quarter.

Table 4-1: Univariate logistic regression to find the potential predictor variables for *R. microplus* and *R. decoloratus* occurrence.

Variable	Description of variable	*R. microplus*		*R. decoloratus*	
		p-value	Coefficient	p-value	Coefficient
alt	Altitude	0.018 *	-0.001	1.13e-08 ***	0.003
Bio_01	Annual mean temperature	0.528	-0.004	5.80e-05 ***	-0.029
Bio_02	Mean diurnal range	1.04e-05 ***	-0.05	0.119	-0.017
Bio_03	Isothermality	1.36e-05 ***	0.341	3.63e-06 ***	-0.359
Bio_04	Temperature seasonality	2.04e-07 ***	-0.003	0.01 *	-0.001
Bio_05	Maximum temperature of the warmest month	0.00339 **	-0.016	0.03 *	-0.011
Bio_06	Minimum temperature of the coldest month	0.001 ***	0.029	0.001 ***	-0.028
Bio_07	Temperature annual range	4.96e-08 ***	-0.037	0.959	0.0003
Bio_08	Mean temperature of the wettest quarter	0.527	-0.004	7.79e-07 ***	-0.035
Bio_09	Mean temperature of the driest quarter	0.521	0.004	0.005**	-0.019
Bio_10	Mean temperature of the warmest quarter	0.183	-0.008	6.39e-05 ***	-0.026
Bio_11	Mean temperature of the coldest quarter	0.623	0.004	0.000294 ***	-0.027
Bio_12	Annual precipitation	6.21e-06 ***	0.003	0.045 *	0.001
Bio_13	Precipitation of the wettest month	7.48e-07 ***	0.013	0.042*	0.005
Bio_14	Precipitation of the driest month	7.78e-05 ***	0.125	0.253	-0.032
Bio_15	Precipitation seasonality	0.479	0.01	0.0004***	0.051
Bio_16	Precipitation of the wettest quarter	1.80e-06 ***	0.005	0.0136 *	0.002
Bio_17	Precipitation of the driest quarter	2.19e-05 ***	0.042	0.166	-0.011
Bio_18	Precipitation of the warmest quarter	1.72e-06 ***	0.004	0.726	-0.0003
Bio_19	Precipitation of the coldest quarter	9.68e-05 ***	0.037	0.404	-0.007
NDVI.max	NDVI maximum value	0.067 .	2.57	0.203	1.688
NDVI.mean	NDVI mean value	0.039*	3.4	0.244	1.862
NDVI.min	NDVI minimum value	0.009 **	5.54	0.466	1.531

*p<0.01, **p<0.001, ***p<0.0001

4.3.2. Multivariate Analysis

4.3.2.1. Rhipicephalus microplus

The stepAIC procedure took 7 steps with AIC values of 306.58 for the first model and 299.52 for the final model. The best model (lowest AIC) for *R. microplus* had the following variables: altitude, annual mean diurnal range, temperature seasonality, minimum temperature of the coldest month, precipitation of the wettest quarter, precipitation of the warmest quarter and the mean NDVI values (Table 2). The AUC of the model was 0.85. For both models (*R. microplus* and *R. decoloratus*), in multivariate analysis, the coefficients have not been included as they are likely to be biased due to

multicollinearity amongst the predictor variables. As already highlighted, the predictive power of the model will not be influenced by multicollinearity. Altitude had the highest contribution in terms of variable importance (22.9%) followed by the minimum temperature of the coldest month (16.4%), mean diurnal range (15.4%), temperature seasonality (14.3%), mean NDVI (13.4%), precipitation of the wettest quarter (10.6%) and lastly precipitation of the warmest quarter (7.6%).

4.3.2.2. Rhipicephalus decoloratus

The stepAIC procedure took 10 steps with AIC values of 373.12 for the first model and 363.25 for the last. The final model for *R. decoloratus* had the following variables: altitude, mean temperature of the driest quarter, annual precipitation, precipitation of wettest month and precipitation seasonality (Table 2). The AUC of the model was 0.73. Altitude had the highest relative contribution to the model (26.3%) followed by the precipitation of wettest month (21.8%), annual precipitation (20.1%), mean temperature of the driest quarter (16.5%) and lastly precipitation seasonality (15.2%).

Table 4-2 : Variables in the suitability models for each tick species after multivariate analysis

R. microplus		*R. decoloratus*	
Variable in the model	Variable Importance / %	Variable in the model	Variable Importance / %
Altitude	22.9	Altitude	26.3
Minimum temperature of the coldest month	16.4	Precipitation of the wettest month	21.8
Mean diurnal range	15.4	Annual precipitation	20.1
Temperature seasonality	14.3	Mean temperature of the driest quarter	16.5
NDVI_mean	13.4	Precipitation seasonality	15.2
Precipitation of the warmest quarter	10.6		
Precipitation of the wettest quarter	7.6		

4.3.3. Habitat suitability maps

The habitat suitability map (Figure 4-2) of *R. microplus* showed a patchy distribution being highly suitable in some parts of the east, north-east and north-west parts of the country. These areas correspond to the Highveld, an area characterised by high average annual rainfall (800-1000mm) and a temperature range of 12-24°C. Although *R. microplus* was collected in the Zambezi Valley and the Middle-veld, the suitability maps show that these areas are not entirely suitable for this tick species. These areas are characterised by low rainfall which negatively affects tick the survival. The Lake Kariba is depicted as suitable for the occurrence of the tick probably because of the moist environment that it provides which is essential for this humid tropical blue tick. Areas adjacent to the lake might be suitable for the establishment of this tick species. The habitat suitability map for *R. decoloratus* (Figure 4-3) shows the tick species occupying almost the entire part of the country with absences in the southern Lowveld and some parts in the east and north east of the country, sites which would be possibly occupied by *R. microplus*. The two maps were combined (Figure 4-4), it is clear from this map that *R. decoloratus* may occupy the greater portion of the country whereas *R. microplus* will be restricted to some pockets in the east and north eastern parts of the country. Other regions in which *R. microplus* has been found mainly in the middle veld and the western Lowveld parts, the tick is not expected to survive in these areas, mainly because of the dry conditions. There are areas in the eastern parts where there is

expected co-existence of the two tick species. The Kendall correlation between the two suitability maps was negative (– 0.18) and highly significant (p-value < 0.001), confirming that both species do not share the same geographical space and tend to be mutually exclusive.

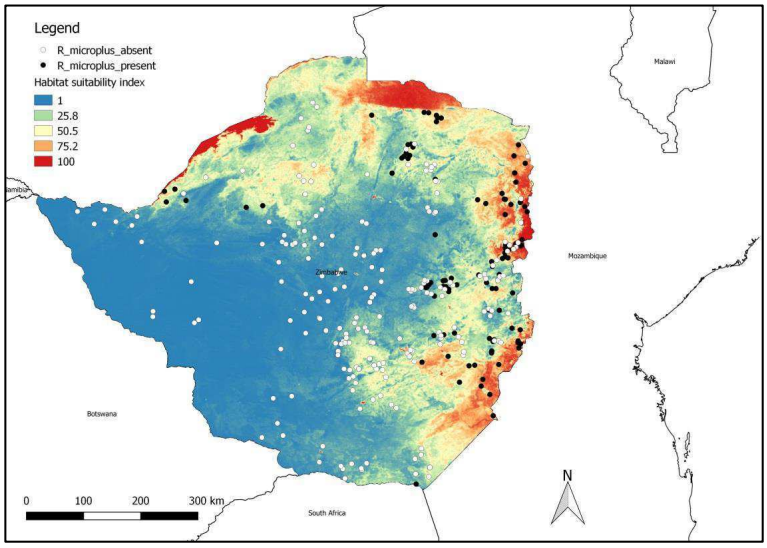


Figure 4-2: Habitat suitability map for *R. microplus* in Zimbabwe

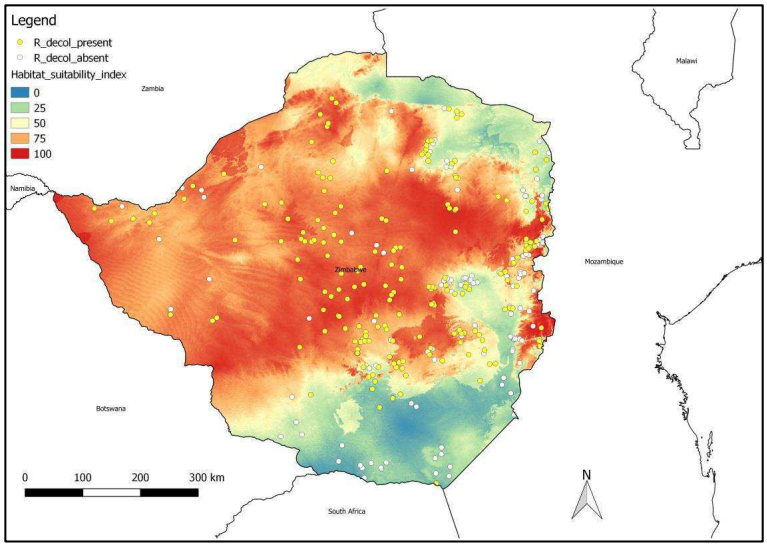


Figure 4-3: Habitat suitability map for *R. decoloratus* in Zimbabwe

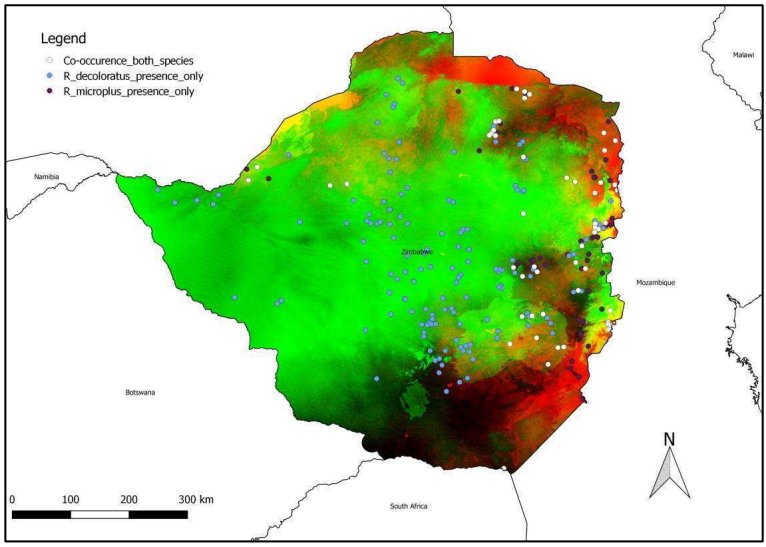


Figure 4-4: Combined habitat suitability map for *R. microplus* and *R. decoloratus* (areas in green are suitable habitats for *R. decoloratus* while areas in red are suitable for *R. microplus*, those in yellow show possible areas of co-existence)

4.3.4. Environmental requirements for the two tick species

The average conditions for rainfall and temperature appear to be similar for both species (Figure 4-5 and Figure 4-6) with peaks observed at temperatures between 18-20°C and rainfall of around 750mm. Rainfall of below 500mm appears unfavourable for both species while an increase of rainfall appears to give *R. microplus* a competitive advantage to *R. decoloratus*. On the temperature gradient, temperatures above ≈ 23°C will tend to favour *R. microplus* at the expense of *R. decoloratus*. Quite interesting is the influence of altitude on the occurrence of the two tick species. In all the models, altitude had the largest contribution (≈22-26%). For both species peak occurrences will occur at altitudes of 1000m above sea level. However lower altitudes tend to favour the proliferation of *R. microplus* at the expense of *R. decoloratus*. On the other hand, the probability of occurrence of *R. decoloratus* at higher altitudes above 1200m is higher than that of *R. microplus*.

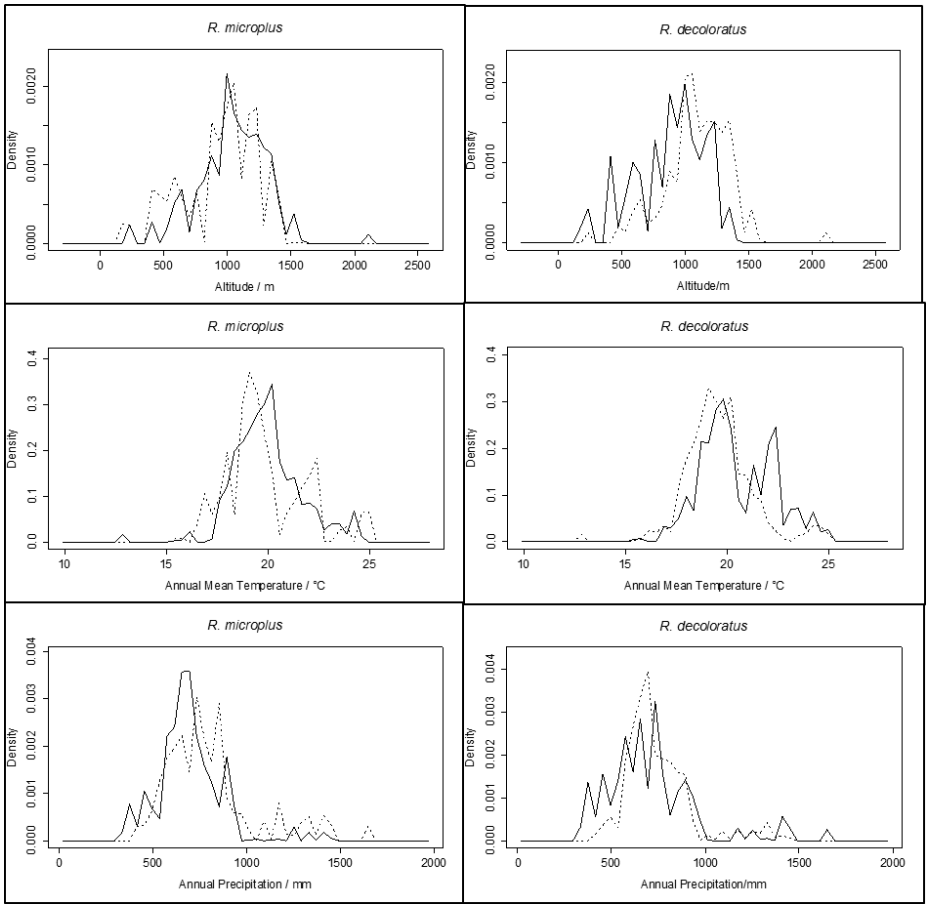


Figure 4-5: Probability density distributions of altitude, annual mean temperature and annual precipitation with respect to the presence (dashed line) and absence (continuous line) of *R. microplus* and *R. decoloratus* in Zimbabwe.

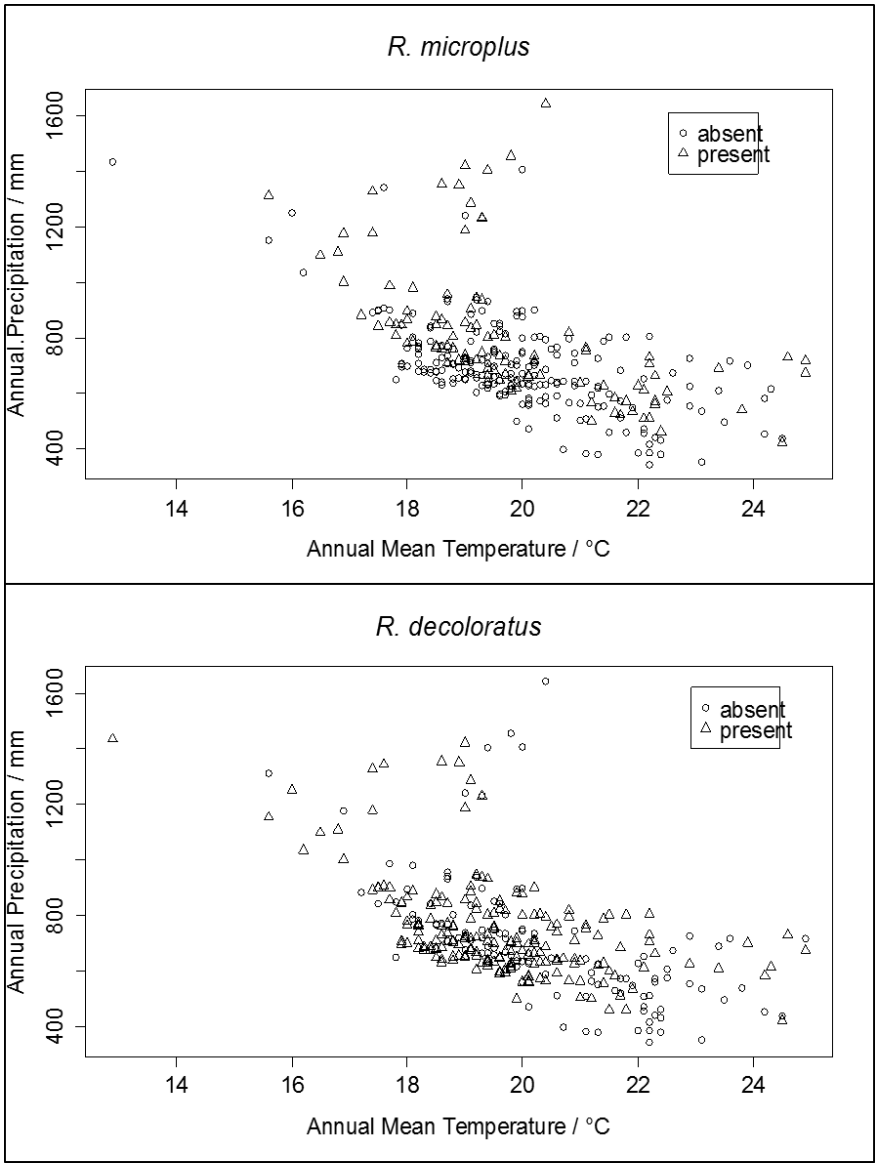


Figure 4-6: Bi-plot showing the conditions for annual temperature and precipitation for *R. microplus*(top) and *R. decoloratus* in Zimbabwe

4.4. Discussion

This study builds upon the efforts made to model the distribution of Boophilid tick species, be it on a global scale (Estrada-Peña et al., 2006a), or on a regional scale in Sub-Saharan Africa (Sutherst and Maywald, 1985), South America (Estrada-Peña, 1999) and West Africa (De Clercq et al., 2013). Countrywide modelling for the Boophilid ticks has been performed in Mexico (Estrada-Peña et al., 2006b), Benin (De Clercq et al, 2015) and Tanzania (Lynen et al., 2008). Cumming (1999) attempted to map the suitable habitats for African ticks including the boophilids of Zimbabwe using records collected between 1975 and 1980. Since then, changes have been reported in Chapter 3. This is the first study to model the distribution of Boophilid ticks parasitizing cattle in Zimbabwe using current records from a nationwide tick survey described in Chapter 3. This information is vital to disease control authorities as it will help in the monitoring of the more pathogenic *Babesia bovis* infections.

In Ixodid ticks, development and mortality rates are governed by temperature and water availability respectively (Estrada-Peña et al., 2015). Correlative modelling, which was used to develop the habitat suitability maps in this study, does not allow examining the specific role played by each climatic variable on the tick life cycle stages (Estrada-Peña et al., 2015). However, patterns are observed which relate to the development and survival of the different life cycle stages of these one-host ticks and these will be discussed. *R. microplus*, as well as *R. decoloratus*, is a one host tick with multiple generations in a year (Walker et al., 2003). Temperature and precipitation variability throughout the year have important implications on the survival of all life stages. Field studies carried out in Zimbabwe indicated that all development stages of both ticks varied, being short, intermediate and longest during the hot, wet and cool seasons respectively (Short et al., 1989b). Diurnal temperature range refers to fluctuations in temperature which will affect tick development and activity on a regional basis with day length accentuating the effects of temperature (James et al., 2015). Tick immature stage development and seasonal activity are influenced by stability in temperature and moisture while extremes of temperature and moisture will influence egg and larvae survival. In field studies to determine the behaviour and survival of unfed ticks Short et al. (1989a) observed that the survival times of *R. microplus*, *R. decoloratus* and *R. appendiculatus* larvae were influenced by low temperature stress in June/July (winter)

and high temperature stress in September/October (hot dry season). Thus minimum temperature of the coldest quarter will have an influence on larval development as it has been observed in South Africa where *R. microplus* larvae were found in vegetation during the winter whilst those of *R. decoloratus* seemed to disappear (Nyangiwe et al., 2011). There is a positive correlation between precipitation and humidity, which is vital for tick survival (Needham and Teel, 1991), especially for Ixodid ticks (Randolph and Storey, 1999).

The variables used in this study represent annual trends and seasonality as well as extreme environmental factors of temperature and precipitation (Estrada-Peña et al., 2013). For *R. microplus,* the variables which had the greatest influence on the model were altitude, minimum temperature of the coldest month, mean diurnal range, temperature seasonality, mean NDVI, precipitation of the warmest quarter and precipitation of the wettest quarter. The variables which had the greatest influence on the model for *R. decoloratus* were altitude, precipitation of the wettest month, annual precipitation, mean temperature of driest quarter and precipitation seasonality. Variability in temperature and precipitation has been observed to drive the distribution of other Ixodidae tick species studied (Hahn et al., 2016; James et al., 2015; Johnson et al.,2016). It is not clear why annual mean NDVI seemed not to influence the occurrence of *R. decoloratus* despite it being viewed as a variable that best captures the distribution of ticks ; it is also considered to be a proxy for water availability which is key to the survival of ticks (Cumming, 2002). In both species, altitude had the greatest contribution to the models, which confirms earlier studies listing altitude as the best single predictor for use in estimating tick distributions (Cumming, 2002).

Looking at the average conditions of temperature and rainfall (annual trends), they appear largely the same for both species while extremes influenced the occurrence of the two species. High and low temperatures favoured *R. microplus* and *R. decoloratus* respectively. The same trend was observed for rainfall where high extremes favoured the occurrence of *R. microplus* whilst low extremes favoured the occurrence of *R. decoloratus*. Similar observations were made in Tanzania by Lynen et al. (2008) when they compared the distributional ranges of *R. decoloratus* and *R. microplus.*

Comparing with earlier modelling papers, the distribution of *R. microplus* seemed to have expanded with more occurrences of the tick in the Zambezi Valley near Lake Kariba in the north western parts of the country. The patchy and discontinuous distribution of *R. microplus* shows that this tick species has established in the east, and the north-eastern parts of the country, in the south-east Lowveld region as well as the environment around Lake Kariba. During the nationwide survey described by in Chapter 3, *R. microplus* was found for the first time in this area when compared to previous studies (Norval et al., 1983, Katsande et al., 1996). The water from the lake does provide the essential humid conditions for the proliferation of this two tick species in the surrounding environment. From the model, there are areas where it is postulated that *R. microplus* and R. *decoloratus* will co-exist. The reasons for the continuous co-existance of *R. decoloratus* with *R. microplus* have been described by in Chapter 3: Persistent droughts may lead to temporary disappearance of *R. microplus* hindering its permanent establishment and subsequent displacement of *R. decoloratus* (Norval et al, 1983). Also tick control practises, where *R. decoloratus* has been found to be more resistant to acaricides (Baker et al., 1981, Mason and Norval, 1980), and adaptability of *R. decoloratus* to wildlife (Sutherst, 1987) may provide *R. decoloratus* with a competitive advantage. The introduction of *R. microplus* as well as the temporary disappearance and emergence may influence the epidemiology of *Babesia bovis.* Norval et al. (1983) found out that in areas where *R. microplus* had been recently introduced there was no endemic stability for babesiosis, which existed in areas where the tick species appeared to be established.

The model output indicates that the habitat suitability of *R. microplus* in Zimbabwe continues to be restricted, having a patchy and discontinuous distribution. *R. decoloratus* still has a wider distribution in the country with very few areas of co-existence between the two species and possible displacement of *R. decoloratus* in areas highly suitable for *R. microplus*. These results suggest that *R. microplus* is not expected to survive in most parts of the country in the event of spread into new areas, for example by cattle movement. The Zambezi Valley might not entirely be suitable for this tick except for areas close to the lake which might provide appropriate humidity conditions for the survival of the tick species. Surveys in the southern province of Zambia did not confirm the presence of *R. microplus* (Speybroeck et al., 2002). It might

be important to resample areas in the southern province of Zambia as well to check if *R. microplus* occurs near the Lake Kariba on the Zambian side.

In conclusion, although other factors could influence the distribution of these two tick species, such as host abundance and tick control practises, models have been produced which seek to explain the limitations in as far as the spread of these ticks is concerned. What would be important is to study the population dynamics of these two species in the different ecological environments. Further, genetic differentiation of *R. microplus* tick populations could be investigated in order to try to understand whether there is local adaptation in the various areas in which the tick species was collected.

Chapter 5: Population structure and genetic diversity of *Rhipicephalus microplus* in Zimbabwe

5.1. Introduction

The use of molecular markers in the study of ticks provides new insights into their population structure and taxonomic relationships (Paulauskas et al., 2006). Investigating the genetic structure of tick populations allows acarologists to answer crucial questions about their biology. This is important because the control of tick-borne diseases (TBDs) is primarily focused on the vector ticks (Giles et al., 2014). Among the factors under investigation are tick dispersal mechanisms, mating patterns and evolutionary adaptations to the environment (McCoy, 2008). It is important to note that such factors will have important implications on the transmission dynamics of pathogens that these ticks carry as vectors, as well as resistance to the acaricide chemicals used to control the ticks (Chevillon et al., 2013).

Chapter 3 showed that the distribution of the one-host tick *Rhipicephalus microplus* in Zimbabwe has expanded, and this was supported by collections from previously unrecorded and ecologically different areas. Of particular concern are the low-lying areas, where temperatures and humidity levels are not favourable for the proliferation of this tick species. The expansion of the geographic range of *R. microplus* could be attributed to cattle movement within and between the different provinces of Zimbabwe. Due to the absence of strict movement controls of livestock, cattle may move from one province to another without being inspected for the presence of diseases or vectors such as ticks. Therefore, cattle carrying ticks or other parasites can move between areas, leading to parasite invasion in previously unoccupied areas. This movement can be in one direction, or it can be bi-directional. One-way movement of cattle together with ticks might result in geographic or genetic isolation of ticks, leading to founder effects that may result in genetic drift. In contrast, bi-directional movement of ticks will result in panmixia, which is characterised by high levels of genetic exchange between populations. All of these scenarios might influence the transmission dynamics of vector pathogens, as well as resistance of vector ticks to acaricides (Chevillon et al., 2013).

An investigation of gene flow between tick populations in Zimbabwe may be helpful to infer cattle movement patterns, which in turn might have led to tick migrations. Additionally, the evolutionary adaptations to different ecological environments of the cattle tick can be investigated. Therefore, the aims of the current study were to investigate genetic differentiation and gene flow patterns in *R. microplus* sub-populations of Zimbabwe. The null hypothesis was that there would be little to no genetic differentiation between geographically close sub-populations, and that differentiation would increase as a function of distance and decreased gene flow.

5.2. Materials and Methods

5.2.1. Biological materials and DNA extraction

Rhipicephalus microplus tick samples were obtained from a nationwide survey conducted as described in Chapter 3. Each province was represented by thirty tick samples, forming provincial populations. Provinces included in the study were Manicaland, Mashonaland Central, Masvingo, Matabeleland North, and Midlands (Figure 5-1). Total genomic DNA was extracted from *R. microplus* ticks using the QIAamp genomic DNA kit (Qiagen, Hilden, Germany).

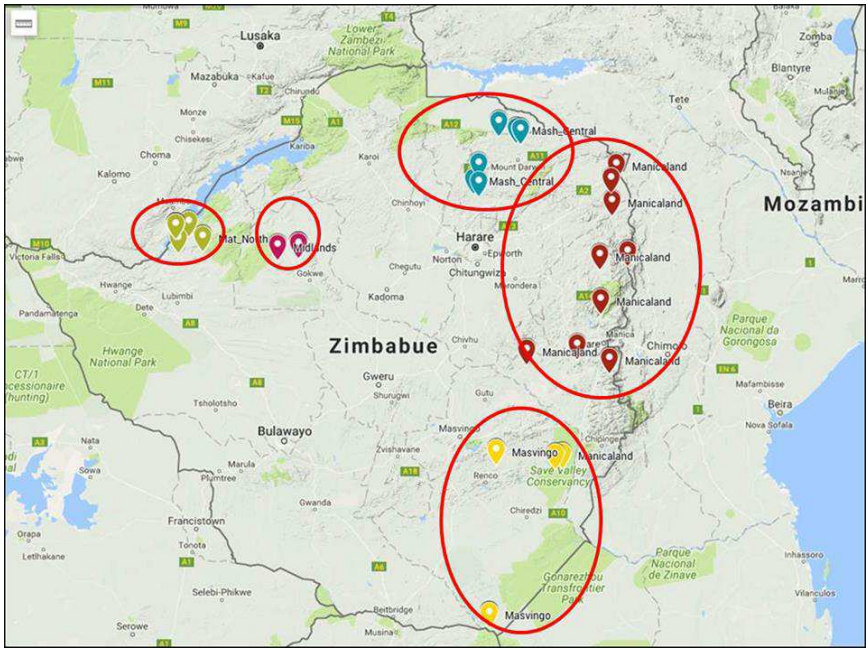


Figure 5-1: Map of Zimbabwe showing the five provincial populations where *R. microplus* samples for this study were obtained.

5.2.2. Microsatellite Selection and data analysis

A total of 27 microsatellite loci were evaluated for their utility to study the population genetics of *R. microplus* in Zimbabwe. Fifteen of these were obtained from the University of Pretoria, Department of Genetics, Ticks and Tick-Borne Disease Research Unit (unpublished), four were described by Chigagure et al. (2000), five by Cutullé et al. (2009), and three by Busch et al. (2014). Thirteen microsatellite loci were chosen based on their PCR efficiency (>75% amplification success), type of repeats, and the presence of polymorphism in a test sample of 11 ticks obtained from each of the five provincial populations. Fluorescently labelled forward primers were used to amplify each locus from each tick sample, and the fragment sizes were determined by the VIB genetic service facility, University of Antwerp. Genotyping was performed using Geneious software (Kearse et al., 2012). Eight loci with average estimates of gene diversity (H_s) greater than 0.6 (Koffi et al., 2006) were selected for further analysis (Table 5-1). These loci were PCR amplified in single-plex for 150 *R. microplus* DNA samples, and analysed

in three panels using an ABI 3730 Genetic Analyser (VIB Genetic Service Facility, University of Antwerp).

The genotyped samples were tested for the presence of null alleles, scoring errors and large allele dropout using the software MICRO-CHECKER v2.2.3 (Van Oosterhout et al., 2004). Linkage disequilibrium (LD) amongst pairs of loci was tested using FSTAT software (Goudet, 2001) based on the log-likelihood ratio G statistic. The same software was used to estimate allelic richness and the average genetic diversity (H_s). Estimations of the mean number of alleles, number of private alleles and Analysis of Molecular Variance (AMOVA) were performed using GenAlex (Peakall and Smouse, 2012). Pairwise F_{ST} (θ) values corrected for sample size (Weir and Cockerham, 1984) were computed using Genodive v2.0b14 (Meirmans, 2009) to compare genetic differentiation amongst populations.

To visualise the geographic clustering of different populations, Principal Co-ordinate Analysis (PCoA) was done using GenAlex. Additionally, a Mantel test was done in GenAlex to show the correlation between genetic and geographic distances. The genetic structure of the population and the likely number of clusters (K) was explored using STRUCTURE v.2.3.4 (Pritchard et al., 2000). All of the genotyped tick samples were included, and the number of potential clusters was set from 1 to 5, with 10 independent runs and a burn-in period of 50 000 iterations followed by 150 000 Markov Chain Monte Carlo (MCMC) iterations. An admixture model with correlated allelic frequencies was used together with a LOCPRIOR model, which takes into account the original population of each tick individual. The most likely number of clusters (K) was inferred by assessing ΔK (Evanno et al., 2005) using STRUCTURE HARVESTER v0.6.94 (Earl, 2012)

Table 5-1: Final list of selected microsatellite loci and their reaction conditions

Locus	Primer Sequences (5´->3´)	Dye	Annealing Temperature/°C	Size range (bp)	Co-loading group #
C39A	F:ATAGAAACACTTAAATCGCATAAC	VIC	53	332(310-342)	1
	R: GTCCCTTTGTTGCCGTTTAG				
P804J	F:TTAACTGGCTGAACATAGGAGGAG	6FAM	54	318 (315-342)	1
	R: CGTGATTTTCCCGAGTTGAT				
P801L	F: AACATCACAGAGGCGGTAATC	PET	55	339 (275-355)	1
	R: TTCGCTCCTCTTTCCTCATTACT				
P801G	F: AACTGCCTTTCCTGTGAGTTCAA	6FAM	58	300 (272-305)	2
	R: CCCGATTCTTGGCCGATCTC				
P804A	F: CCAAGCGATAACACATGTATAGG	VIC	55	332 (199-343)	2
	R: GACAGCAAAATCCCGAAGAT				
P804G	F: CTCTATTTTCCCTTAGTGCTCAA	NED	54	345 (295-363)	2
	R: TCAGAAAGAAGCCTACTGATG				
P807F	F: GCCACAAAGCTCGACCTAACTA	VIC	58	322 (315-333)	3
	R: GACTGGGTTAACTGGCGGAACAA				
C27A	F: TCTGACGATACCCCGAACTACAT	NED	55	344 (320-348)	3
	R: TACTACCGCGACAAGCACAATGA				

5.3. Results

5.3.1. Microsatellite selection and data analysis

Only 87 of the 150 samples (58%) resulted in positive amplification for the eight loci (Table 5-2). The number of alleles ranged from 5 to 25 per locus, with an allelic richness of 4-13. The levels of genetic diversity amongst the loci (He) were relatively high (0.6-0.9), while the F_{IS} values were relatively low for all the loci except locus C39A. The MICRO-CHECKER results showed homozygous excesses in all the loci except P801G, while no evidence of scoring errors and large allele dropouts was seen in all the loci except locus C39A, which showed potential scoring errors due to stuttering. Upon further analysis of the peak sizes at this locus, stuttering appeared unimportant, hence this locus was retained. No LD was observed (P<0.001) amongst pairs of loci, signifying that they are statistically independent, and thus these loci were considered suitable for further population genetic analyses.

Table 5-2: Characteristics of loci used in this study across all populations

Locus	Na	Ne	AR	Ho	Hs	F_{IS}
P804J	5	2.57	4.57	0.35	0.62	0.37
C39A	9	3.38	5.57	0.35	0.73	0.49
P801L	14	6.78	10.11	0.59	0.83	0.30
P801G	10	5.82	8.32	0.78	0.85	0.05
P804A	25	7.48	13.06	0.57	0.90	0.33
P804G	17	8.23	11.20	0.76	0.91	0.14
P807F	7	4.47	6.44	0.67	0.79	0.12
C27A	8	3.71	5.96	0.60	0.76	0.18

Na = No. of Alleles, Ne = No. of Effective Alleles , AR = Allelic Richness, Ho = Observed Heterozygosity , Hs = Average estimate of within sample gene diversity F = Fixation Index.

5.4. Genetic differentiation and population structure

The median genetic diversity (He) was 0.763 (0.755-0.802) with Matabeleland North having the lowest genetic diversity (Figure 5-2). Manicaland had the highest number of private alleles as compared to other populations (Figure 1). The AMOVA analysis revealed that 97% of the genetic variation existed within populations, while 2% of the genetic variation existed between populations (F_{ST}= 0.023, *P*<0.001, Table 5-3).

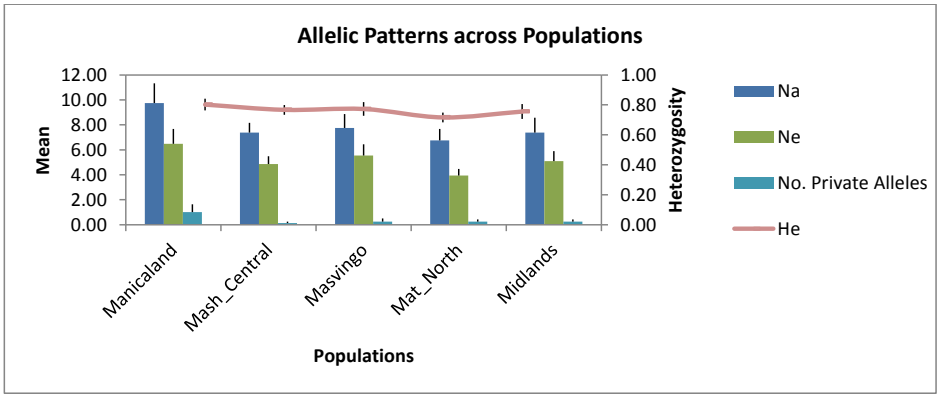


Figure 5-2. Distribution of allelic patterns between populations (Na = No. of Different Alleles, Ne = No. of Effective Alleles, No. Private Alleles = No. of alleles unique to a single population, He = Expected Heterozygosity).

Table 5-3: AMOVA for the different populations (df = degrees of freedom, SS = sum of squares, MS = mean square)

Source	df	SS	MS	Variance Component	% of Total Variance / %
Among Populations	4	27.161	6.790	0.107	3%
Within Populations	169	537.667	3.158	3.158	97%
Total	173	560.828		3.265	100%

There was little to no genetic differentiation amongst the populations. However, the pairwise F_{ST} (θ) values were significant at the 5% level amongst all pairs of populations, except between Masvingo and Manicaland, and between Masvingo and Midlands (Table 5-4). This observation was supported by the PCoA analysis (Figure 5-3), which did not show an obvious clustering of populations, except for partial clustering of samples from Matabeleland North. These results were further corroborated by the Mantel test, which did not show significant patterns of isolation by distance (IBD) among the different populations (*P*=1.000). The correlation between geographic and genetic distance was very low (*r*=0.078) (Figure 5-4).

Table 5-4: Pairwise F_{st} (θ) amongst different populations calculated according to Weir and Cockerham (1984) and adjusted for sample size. F_{ST} (θ) values in bold are significant at the 5% level.

	Manicaland	Mashonaland Central	Masvingo	Matabeleland North
Mashonaland Central	**0.015**			
Masvingo	0.000	**0.028**		
Matabeleland North	**0.059**	**0.076**	**0.060**	
Midlands	**0.023**	**0.025**	0.011	**0.041**

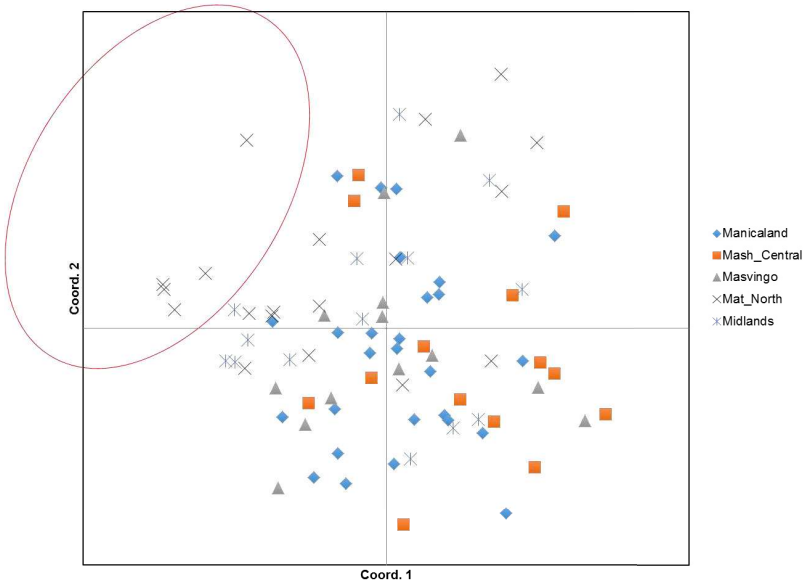


Figure 5-3: Principal Co-ordinate Analysis (PCoA) of genotypes of samples originating from the five provinces where *R. microplus* was found.

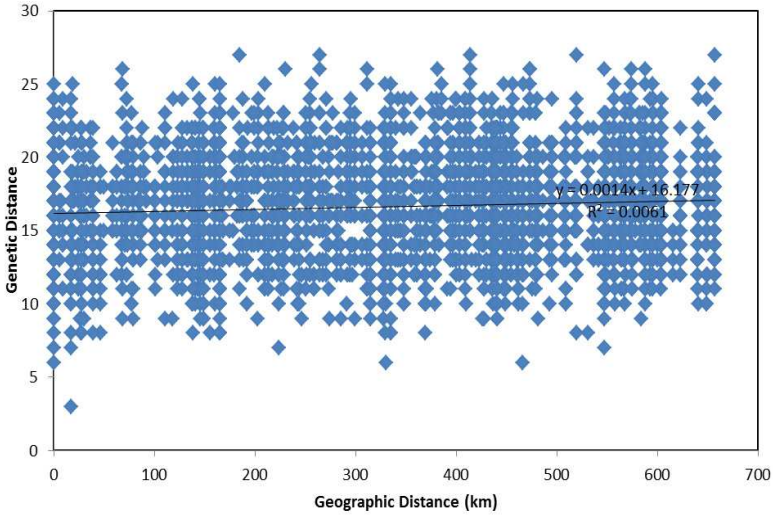


Figure 5-4: Analysis of Isolation by Distance (IBD) showing correlation between geographic distance and the genetic distance between the *R. microplus* samples.

STRUCTURE analysis suggested that the probable number of *R. microplus* populations in Zimbabwe was K=2, with ΔK=32.8. No ΔK values were reported for K=1 and K=5, while for K=3 and K=4 the ΔK values were 1.7 and 0.02 respectively, confirming that there were two genetically distinct clusters (Figure 5-5).

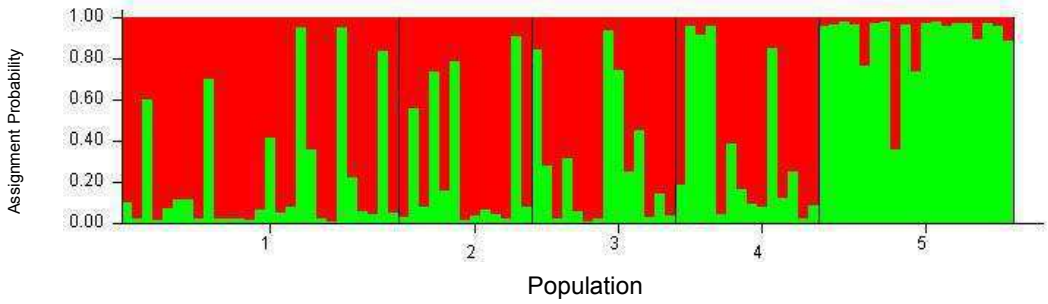


Figure 5-5: *Rhipicephalus microplus* tick population structure (1- Manicaland, 2-Masvingo, 3-Mashonaland Central, 4-Midlands, 5-Matabeleland North).

5.5. Discussion

The study revealed high levels of genetic diversity within *R. microplus* populations and little genetic differentiation amongst them. There was high allelic diversity amongst the loci, despite an excess of homozygotic loci. The source population of *R. microplus* in Zimbabwe, namely the Manicaland province, had the largest number of private alleles and genetic diversity. The results of the study suggest an infinite island population structure model for *R. microplus* in Zimbabwe, which is in migration-drift equilibrium. This was supported by population structure analysis, which showed admixture in all the sub-populations, although the recent range expansion in Matabeleland North was suggestive of founder effects.

The high levels of genetic diversity observed in this study indicate increased gene flow within and among populations. High levels of within-population genetic variation and weak genetic structure between populations has been observed in other ixodid tick species (Delaye et al., 1997; Kanduma et al., 2015; McCoy et al., 2012). This was attributed to high dispersal rates amongst host species, resulting in genotype mixing and panmixia. These movements will indirectly facilitate dispersal of ticks. In Kenya, a weak genetic structure in cattle amongst different populations was observed and this was attributed to extensive movement of cattle for socio-cultural exchange and trading purposes (Rege, 2001) and subsequently explained the weak genetic structure observed amongst *Rhipicephalus appendiculatus* tick populations (Kanduma et al., 2015). Increased gene flow amongst populations may lead to a spread of acaricide resistant alleles (Beesley et al., 2017), but the lack of genetic differentiation means that there will be no genetic barriers to tick control programmes (Gooding, 1996).

Alleles may be lost as parasites move between populations, resulting in founder effects (Balloux and Lugon, 2002). This phenomenon was observed in the current study, since the source population in Manicaland had the highest number of private alleles as compared to the other populations. This could further result in genetic drift (Roderick and Navajas, 2003), which can be an indicator for local adaptation (Gandon and Michalakis, 2002). This could explain why the population in Matabeleland North appeared to be partially clustered, as seen by the results of the Principal Co-ordinate and STRUCTURE analyses. Although our results indicated that there was no IBD, thus

suggesting migration-drift equilibrium (Kanduma et al., 2015), the low levels of genetic differentiation amongst the populations were significant. Apart from frequent dispersal between established populations, this could also suggest recent population expansion (*cf.* McCoy et al. 2003). However, for the sub-populations that share borders such as Manicaland and Masvingo, and Masvingo and Midlands, the differentiation was insignificant. This tended to partially support the null hypotheses, which was that differentiation would increase as a function of distance and decreased gene flow. The total absence of genetic differentiation between Masvingo and Manicaland is a result of the bi-directional movement of cattle as will be explained in the next Chapter.

The excess homoyzgotes observed in the microsatellite loci were not attributed to the presence of null alleles, since only positive samples were sent for fragment size analysis after PCR amplification in single-plex. The occurrence of these homozygote excesses could rather be attributed to the Wahlund effect as a result of the inadvertent pooling of individuals from different populations (Dharmarajan et al., 2011). Alternatively, this could be attributed to the biology of the tick at the infra-population level, where development occurs simultaneously within large brotherhoods of individuals, which go on to seek hosts as a group, develop to adults simultaneously, and mate with each other (Koffi et al., 2006). This results in inbreeding and increased homozygosity (*cf.* Dharmarajan et al., 2011).

Unrestricted cattle movement may be responsible for the frequent gene flow amongst the different *R. microplus* tick populations leading to weak population structure. However it is observed that alleles are lost as ticks migrate from the source population. The consequences of such allele losses have not been clearly observed in the current study. Thus, it will be important to study the phenology of *R. microplus* in these ecologically different habitats, and compare those results with the genetic diversity in order to understand local adaptation.

Chapter 6: Communal farmers' perceptions of tick-borne diseases affecting cattle and investigation of tick control methods practiced in Zimbabwe

This chapter is based on: Sungirai, M., Moyo, D.Z., De Clercq, P. & Madder, M. (2016) Communal farmers' perceptions of tick-borne diseases affecting cattle and investigation of tick control methods practiced in Zimbabwe. Ticks and Tick Borne Diseases, 7, 1–9

6.1. Introduction

Ticks and tick-borne diseases (TBDs) are one of the major constraints to livestock production in the (sub) tropical areas of the world (Jongejan and Uilenberg, 2004). Global economic losses due to ticks and tick-borne diseases have been conservatively put at US$18.7 billion annually (De Clercq et al., 2012). The losses are incurred through the direct effects of ticks as blood sucking parasites and indirect effects as disease vectors which will lead to reduced growth rate, fertility problems, decline in milk production, reduced value of hides and livestock mortalities, notwithstanding the costs associated with treatment and control (Minjauw and Mcleod, 2003). The best way to control TBDs is through the control of the vector ticks (Willadsen, 2006) and various strategies have been proposed (Jonsson, 2004; Pegram et al., 2000; Peter et al., 2005). Historically, in most countries, particularly in Africa, the control of ticks and other vectors has been the responsibility of veterinary departments financed by the government but this responsibility has been transferred to livestock owners due to economic structural adjustment programs (Peter et al., 2005). However, this development has led to a widening gap between the veterinary services and livestock owners such that veterinarians and other animal health professionals no longer engage farmers to learn about their views, their problems and disease priorities (Mariner et al., 2011).

Since livestock owners are now playing a pivotal role in the control of TBDs, it becomes important to investigate the farmers' perceptions on the constraints they face and the benefits of the different technologies they use to solve those constraints (IFAD, 2004). This could be best achieved by conducting participatory epidemiological surveys where farmers are involved in defining and prioritizing veterinary related problems and also identifying and developing solutions to those problems (Catley et al., 2012). Studies of this nature will lead to more effective management of livestock diseases. This is so because the priorities of the farmers might be different from the priorities of the national veterinary services and they should be taken into account in the implementation of livestock disease control programs (De Garine-Wichatitsky et al., 2013).

The livestock sector in Zimbabwe is composed of large scale commercial, small scale commercial, the A1 and A2 resettlement as well as the communal farmers (Mavedzenge

et al., 2006). The large scale, small scale and resettlement farmers are involved in intensive livestock production for profit. The A1 and A2 resettlement farmers are those that have benefited from the land reform program, with the latter farming on a large scale while the former are predominantly small scale farmers. Farming in the communal sector is largely for subsistence purposes with occasional selling of surplus in times of emergencies. It is important to note, however, that communal farmers in Zimbabwe own the majority of the cattle at more than 80% (Tavirimirwa et al., 2013).

Historically the most important tick borne diseases in the country are cowdriosis, babesiosis, anaplasmosis and theileriosis. The epidemiology of these diseases has been studied in the past (Katsande et al., 1999; Latif et al., 2001; Norval et al., 1984; Peter et al., 1998b). Tick-borne diseases are responsible for more than 60% of all cattle mortalities in the country (DVS, 2013). This has led to the government playing a central role in tick control programs in communal areas and A1 resettlement areas, where they co-ordinate the purchase and supply of acaricides. Communal and A1 resettlement farmers are required to pay a fee of USD$2 per animal annually so that they participate in these government initiated programs. In large scale, small scale and A2 resettlement schemes it is largely the prerogative of the farmers to take their own initiatives when it comes to tick control.

In communal and A1 resettlement areas tick control is primarily based on the use of the plunge dip where communal farmers who would have paid dipping fees bring their cattle to a centrally located dip tank and have them submerged in a dip wash with acaricides, which is commonly referred to as ‘dipping’. The reduction in government financial subsidies in tick control has been seen to change the attitudes and perceptions of farmers with regards to tick control programs (Pegram et al., 1993). It will be important then to understand whether farmers perceive TBDs the way the government does, investigate their level of participation in tick control programs and their own interventions in as far as tick control is concerned. As highlighted earlier, participatory surveys will be helpful in soliciting such kind of information which will be very useful in sustainable disease control programs (Masika et al., 1997).

Communal farmers can have a large wealth of indigenous knowledge on livestock diseases which could be viewed as a natural extension of the veterinary diagnostic

service (Catley et al., 2002). Disease control programs often have failed because the local farmers have not been involved in identifying their problems and selecting, testing and evaluating possible solutions (Minjauw et al., 2002). According to (Chenyambuga et al., 2010) the currently held concept of TBDs control has to be revised and should consider the indigenous knowledge of livestock keepers. Literature search on the involvement of farmers in studying the epidemiology of diseases in Zimbabwe revealed that only a few studies had been conducted (Chikerema et al., 2013; De Garine-Wichatitsky et al., 2013; Mosalagae et al., 2011) and none of these have directly looked at TBDs. This is despite the importance placed on TBDs by the government Department of Veterinary Services in the country. Hence the purpose of this study was to investigate the perception of communal farmers with regards to TBDs, level of participation in government initiated tick control programs, extent of practicing other tick control methods and classes of acaricides used. The influence of age, gender, level of education, farmer training and problems of TBDs in the area on the awareness of TBDs was also investigated.

6.2. Materials and Methods

6.2.1. Study area

The study was carried out in Bikita, a district in Masvingo province of Zimbabwe (Figure 6-1) between February and April 2014.There are three distinct ecological regions in the district (Chikodzi et al., 2013). The north-western part falls under ecological region 3 at an altitude of between 500-1000m above sea level with an average annual rainfall of 650-800mm. The temperature ranges from 18-24°C. The south-western, central and north eastern part falls under ecological region 4 and this region dominates most of Bikita district. Average rainfall is 400-640mm per year with an altitude above sea level of 450-900m. Seasonal droughts are common. The temperature ranges from 20-25°C.The extreme south and south-eastern part falls under ecological region 5. The average annual rainfall is 300-500mm with an altitude above sea level of 450m-500m. The climate is very hot with a mean temperature range of 22-30°C. Ecological region 5 of Bikita is largely composed of the Save Valley Conservancy with large tracts of forestry and wildlife areas and low cattle densities and or absence of cattle.

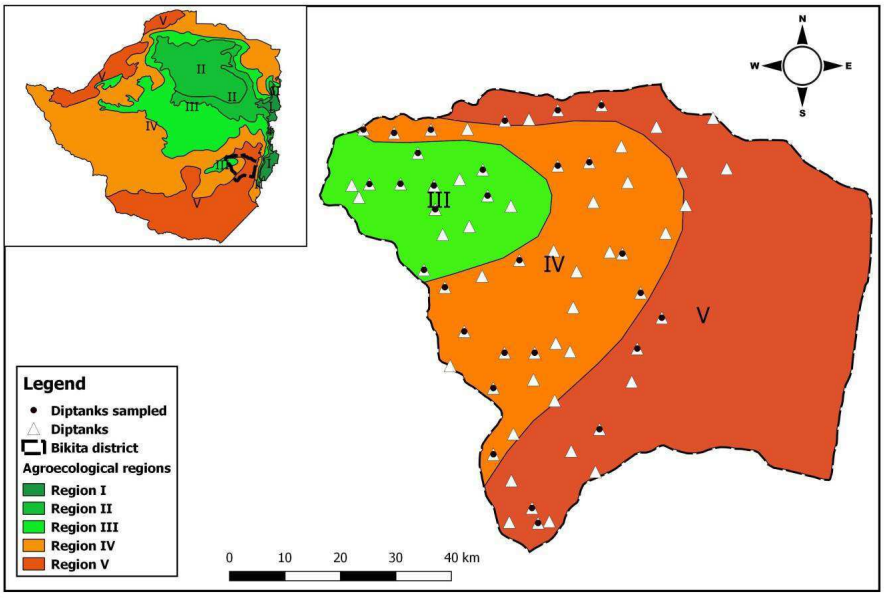


Figure 6-1: Map showing the location of Bikita district together with the dip tanks sampled (questionnaire administration and tick collection) within each ecological regions and the total number of dip tanks in the district.

6.2.2. Data collection

The study was designed to be carried out as personal interviews. Informal discussions were first carried out with key informants in the district on general animal health issues and tick-borne diseases in particular. The key informants included the animal health inspectors, livestock specialists, extension officers, village heads, school teachers. Thereafter a questionnaire was designed and tested amongst veterinary assistants and 30 farmers who were randomly selected in the district. After this initial exercise the questionnaire was re-designed taking into account the inputs and modifications that were identified during the pre-testing stage. The interviewers were selected from local veterinary assistants who were trained in the administration of the questionnaire to solicit information without bias.

The personal interviews were planned such that they occurred on dipping days. It was believed that this was not a significant source of selection bias since most if not all of

the communal farmers are expected to bring their cattle to the dip (de Garine-Wichatitsky et al., 2013). Dipping sessions are conducted by a dip attendant who is employed by the government and ensures that dipping procedures are followed. The dip attendant works closely with the local Livestock Development Committee (LDC) which is comprised of selected members of the village who are responsible for ensuring among other things that water is fetched in nearby dams, rivers or wells and put into the dip, and that villagers pay dipping fees. Whenever there are important issues to be discussed by the community this is done during the dipping days as then most farmers would be available. Therefore dipping sessions provide a platform for easy access to cattle owners. Hence for the purposes of this study, the dip attendant together with LDCs were asked to inform farmers of special meetings during dipping days in order to facilitate the conducting of interviews.

The type of information gathered included age, sex, level of education, farmer training, knowledge of ticks and tick-borne diseases, ranking of the most important tick-borne and tick related disease according to the farmer. Farmers were asked if they knew about TBDs, and if they did they were asked to give names of any TBDs they knew or describe clinical or post-mortem signs. The name of the disease was only inferred when typical clinical and post-mortem signs were observed by the farmers. In the case when atypical clinical and post-mortem signs were given, no disease was assigned. Farmers were asked also on the consistency of using the plunge dip: if farmers had last brought their cattle for dipping at the most two weeks before they were regarded as consistent, otherwise if it was more than two weeks they were not considered consistent. The use of other tick control methods practiced apart from the plunge dip was also investigated. Other questions asked included the types of acaricides used (farmers were asked before the meeting to bring sachets of the chemicals they use for tick control if ever they had any), views on acaricide resistance (farmers were asked if they would see any changes in the number of ticks on the animal after application of acaricides), trends of tick-borne diseases over the past 5 years, cattle losses due to tick-borne diseases in the past three months and the person who confirmed the diagnosis in the event of cattle deaths.

6.2.3. Sampling

Multi-stage sampling was done with the household as the primary sampling unit whilst the dip tank was the secondary sampling unit. Information on the number of households with stock cards (proof of ownership of cattle) visiting a dip tank was obtained from the district office of the department of Veterinary Services and it was observed that this ranged from 100-150. Taking the upper threshold of 150 households, the total number in all the 68 operational dip tanks in the area was estimated at 10 200. The formula described by Chatikobo et al., (2013) was used to calculate the number of households to be sampled assuming (no prior knowledge) those with problems of ticks and tick borne diseases were 50% of the population. Using this formula 408 households had to be sampled. The dip tanks are located in three different ecological regions with 14 (region 3), 34 (region 4), and 22 (region 5). Assuming (no prior knowledge) that ticks were a problem in 50% of the dipping tanks the formula of Thrusfield (2005) was used to calculate the number of dip tanks to be sampled and these were 14 (region 3), 32 (region 4) and 20 (region 5). Thus a total of 66 dip tanks had to be sampled with an average of 7 households per dipping tank. This would proportionally increase by the number of dipping tanks in each ecological region. Dip tanks in each ecological region were assigned numbers. Random numbers as many as the number dip tanks in each ecological region were generated using MS Excel 2007. Dip tanks were to be visited according to the order of random numbers. It was also decided to sample randomly at least 10 households instead of seven. Due to circumstances which have been described in Chapter 3 and the fact that the study largely relied upon successful mobilisation of study participants to visit the dip tank, not all the dip tanks which had been initially targeted were sampled. Thirty one dip tanks were visited eight in region three, 15 in region four and eight in region five giving a total of 31 dip tanks. A total of 313 household representatives with stock card holders were interviewed in this study. Independent observations on the dipping process, discussions with key informants and members of the LDCs on their approach towards tick control, role of government extension services in disease control, problems faced and possible solutions to those problems was done.

6.2.4. Data analysis

Information gathered from the questionnaire was collated and entered into SPSS version 21 for descriptive and statistical analysis. Not all the farmers were able to name the disease but some did give clinical or post mortem signs. The pattern of signs given by the farmers was observed and this was put into coherent categories such as, “bloody urine, hard dung, teat damage, swollen udder, nervous symptoms in the case of farmers saying cows appeared mad, emaciation,” etc. When the signs given were typical of a TBD or any other disease, signs were attributed to that disease e.g., “heartwater signs”. However, when the signs were atypical no name was assigned for a disease and the description was e.g., “loss of appetite”.

The non-parametric Friedman test was used to rank the most common diseases mentioned by farmers. Scores were given for the diseases as they had been listed by farmers. A score of 1 was given to the disease first on the list whilst diseases furthest from the list were given higher scores. The chi-square test was used to determine the significance of associations between the knowledge of TBDs as well as use of other tick control methods with demographic factors (sex, age, level of education and basic farmer training) together with variables such as problems of TBDs in the area and claim of knowledge of TBDs. All statistical tests were done at the 5% level of significance.

6.3. Results

6.3.1. Demographics and summary of major responses

The demographics of the population under study are shown in Table 6-1. More males were interviewed as compared to females. Most respondents were above the age of 40. The majority of the people had attended basic education with a low number admitting to never have gone to school. Most of the farmers interviewed had not participated in basic farmer training. Furthermore, Table 6-1 shows the responses of farmers to various questions asked during the study. The majority of the farmers reported problems of ticks and tick-borne / related diseases. Most farmers were consistently following the dipping regime recommended by the Department of Veterinary Services. In addition to that they also practiced other tick control methods with hand spray by a knapsack being the most commonly used method. Of the acaricides used,

the formamidines group was the most common while the organophosphates were the least common. A large proportion of farmers viewed these acaricides as being effective in controlling ticks. Few farmers had lost cattle due to tick-borne / related problems in the previous 3 months. Of those that lost cattle to TBDs the majority of the diagnosis was confirmed by local veterinary officers while in some cases farmers did their own diagnosis or relied on colleagues. There were mixed responses as to whether TBDs were increasing in the district although a large proportion were of the view that the incidence of the diseases was increasing.

Table 6-1: Demographics and summary of major responses

Factor	Level	Respondents (%)	Lower limit 95% Confidence Interval/%	Upper limit 95% Confidence Interval/%
Gender	Male	64.6 (197/305)	59.23	69.97
	Female	35.4 (108/305)	30.03	40.77
Age	18-25	9 (28/312)	5.82	12.18
	26-30	11.5 (36/312)	7.96	15.04
	31-40	26 (81/312)	21.13	30.87
	+40	53.5 (168/312)	47.97	59.03
Level of education	Primary	30.1 (94/312)	25.02	35.18
	Secondary	43.6 (136/312)	38.11	49.09
	Advanced Level	4.2 (13/312)	1.98	6.42
	Certificate (1 year of study)	6.1 (19/312)	3.45	8.75
	Diploma (3 years of study)	4.2 (13/312)	1.98	6.42
	Bachelors	2.9 (9/312)	1.04	4.76
	Post-Bachelors (Masters)	1 (3/312)	-0.10	2.10
	None	8.3 (26/312)	4.99	11.01
Basic farmer training course	Yes	39.7 (124/312)	34.28	45.12
	No	60.3 (188/312)	54.88	65.72
Problem with ticks and TBDs	Yes	86.6 (271/313)	82.83	90.37
	No	13.4 (142/313)	9.63	17.17
Claim of knowledge of ticks and TBDs	Yes	71.3 (219/307)	66.28	76.39
	No	28.7 (88/307)	23.61	33.72
Last time dipping done	One week ago	22.7 (71/313)	18.06	27.34
	Two weeks ago	47.6 (149/313)	42.07	53.13
	One month ago	19.5 (61/313)	15.11	23.89
	More than a month ago	10.2 (32/313)	6.85	13.55
Alternative tick control methods	Yes	79.6 (249/313)	75.14	84.06
	No	20.4 (64/313)	15.94	24.86
Tick control methods	Pour on	4.5 (14/313)	2.20	6.80
	Hand spray (Knapsack)	67.4 (211/313)	62.21	72.59
	Smear (Tick grease)	2.2 (7/313)	0.57	3.83
	Traditional methods	5.4 (17/313)	2.90	7.90
	Hand dressing	16.6 (52/313)	12.48	20.72
Type of acaricide used	Formamidines	59.4 (186/313)	53.96	64.84
	Pyrethroids	29.1 (91/313)	24.07	34.13
	Organophosphates	4.5 (14/313)	2.20	6.80
	Macro-cyclic lactones	12.8 (40/313)	9.10	16.50
Effectiveness of acaricides (viewed by the presence/absence of high tick loads after application of acaricide)	Yes	75.2 (231/313)	70.63	80.17
	No	17.3 (53/313)	11.13	19.07
	No idea	9.5 (29/313)	6.25	12.75
Loss of cattle due to ticks in past 3 months	Yes	23.8 (74/311)	19.07	28.53
	No	76.2 (237/311)	71.47	80.93
Confirmation of diagnosis when cattle had died (directed at those who had lost cattle due to ticks)	Self	14.3 (42/313)	10.42	18.18
	Veterinary Officer	18.4 (54/313)	14.11	22.69
	Colleagues	9.6 (28/313)	6.34	12.86
	No response	60.3 (189/313)	54.88	65.72
View of ticks in past 5 years	No increase	53.4 (167/313)	47.87	58.93
	Increase	40.6 (127/313)	35.16	46.04
	I don't know	6.1 (19/313)	3.45	8.75

6.3.2. Perception of ticks, tick-borne and related diseases by communal farmers

A total of 71.3% (219/307) farmers claimed to have knowledge of TBDs, of these 74.4% (163/219) gave the actual names of the diseases and 22.4% (49/219) related TBDs with clinical / post-mortem signs. A total of 40 different classifications were related to TBDs by farmers and these included diseases, clinical and post-mortem signs. If these followed similar patterns, they were grouped and 30 different classifications were used for analysis by the Friedman test. The results of the frequency of mention and ranking are shown in Table 6-2. According to farmers, cowdriosis, mastitis , anaplasmosis, body damage, babesiosis and poor body condition were the most frequently mentioned effects of ticks on cattle. Some farmers managed to describe TBDs' clinical or post-mortem signs and the most common frequently mentioned were "animal moving in circles, water in chest cavity, red urine and hard dung". Direct effects of ticks on cattle were frequently highlighted by farmers and these were, general body damage with tick wounds on most parts of the animal, udder and teat damage, ear damage and abscesses.

Table 6-2: Farmers' views on effects of ticks on cattle (diseases and clinical / post-mortem signs)

	Frequency of mention (expressed as percent of total respondents, n=313)	Lower limit 95% confidence Interval/%	Upper limit 95% confidence Interval/%	Mean rank score (Friedman test)
Heartwater (Cowdriosis)	38 (n=119)	32.62	43.38	11.15
Mastitis	36.7 (n=115)	31.36	42.04	11.17
Gall sickness (Anaplasmosis)	36.1 (n=113)	30.78	41.42	11.53
Body damage (general)	28.4 (n=89)	23.40	33.40	12.50
Red water (Babesiosis)	24.6 (n=77)	19.83	29.37	13.19
Poor body condition	16.6 (n=52)	12.48	20.72	14.35
Mastitis signs (swollen udder, decline / cessation of milk)	14.4 (n=45)	10.51	18.29	14.63
Ear damage	8.6 (n=30)	5.49	11.71	15.47
Heartwater signs (groaning, animal moving in circles, water in chest cavity(post-mortem), twitching of eyes)	8.9 (n=28)	5.75	12.05	15.49
Red water signs (red urine)	8 (n=25)	4.99	11.01	15.73
Abscesses	7 (n=22)	4.17	9.83	15.81
Udder teat damage	5.8 (n=18)	3.21	8.39	15.93
Gall sickness signs (hard dung)	5.4 (n=17)	2.90	7.90	16.02
January disease (Theileriosis)	5.1 (n=16)	2.66	7.54	16.13
Sweating sickness	4.2 (n=13)	1.98	6.42	16.22
Loss of appetite salivation	2.9 (n=9)	1.04	4.76	16.42
Foot rot	2.6 (n=8)	0.84	4.36	16.47
Joint ill	2.6 (n=8)	0.84	4.36	16.47
Sweating sickness signs (hair loss in calves)	2.2 (n=7)	0.57	3.83	16.51
Loss of appetite	2.2 (n=7)	0.57	3.83	16.52
Large and small swollen lumps in body	1.9 (n=6)	0.39	3.41	16.53
January disease signs (swollen lumps on neck)	1.3 (n=4)	0.05	2.55	16.63
Foot rot signs (sores in inter-digital spaces)	0.6 (n=2)	-0.26	1.46	16.74
Blindness	0.6 (n=2)	-0.26	1.46	16.74
Blood stained dung	0.6 (n=2)	-0.26	1.46	16.75
Swollen joints	0.6 (n=2)	-0.26	1.46	16.75
Salivation	0.3 (n=1)	-0.31	0.91	16.79
Dyspnea	0.3 (n=1)	-0.31	0.91	16.79
Loss of appetite and groaning	0.3 (n=1)	-0.31	0.91	16.79
Lumpy skin disease	0.3(n=1)	-0.31	0.91	16.80

6.3.3. Factors influencing knowledge of TBDs and use of other tick control methods

Table 6-3 shows the factors influencing knowledge of TBDs and use of other tick control methods. The ability of the farmers to name a TBD was influenced by level of education, participation in a farmer training course, problems of TBDs in the area and claim of knowledge of TBDs affecting cattle in the area. The ability to name TBDs increased with improved level of education, attendance at a farmer training course, increased problems of TBDs in the area. Farmers who claimed knowledge of TBDs were able to name the TBDs affecting cattle while a low proportion of farmers who claimed no knowledge of TBDs went on to list these as TBDs. More males were able to describe the signs of TBDs as compared with females while those who claimed to have knowledge of TBDs managed to describe the signs. Surprisingly a few of the farmers who claimed no knowledge of TBDs were able to describe the signs of TBDs. The use of other tick control methods was significantly influenced by farmer sex, attendance at a farmer training course and claim of knowledge of TBDs.

Table 6-3: : Factors influencing knowledge of TBDs, and use of other tick control methods

Factor	Level	Ability to name TBD (%=yes)	Ability to describe TBD signs (% =yes)	Use of other tick control methods (%=yes)
Age	18-25	60.7 (17/28)	3.6 (1/28)	78.6 (22/28)
	26-30	63.9 (23/36)	13.9 (5/36)	83.6 (30/36)
	31-40	51.9 (42/81)	14.8 (12/81)	76.5 (62/81)
	+40	55.1 (92/167)	22.8 (38/167)	80.2 (134/167)
		χ^2=1.775, p=0.620	χ^2=7.492, p=0.058	χ^2=0.83, p=0.842
Gender	male	56.9 (112/197)	22.8 (45/197)	83.2 (164/197)
	female	53.7 (58/108)	10.2 (11/108)	73.1 (79/108)
		χ^2=0.280, p=0.596	χ^2=7.456, p=0.006**	χ^2 = 4.394, p=0.036*
Level of education	Primary	47.9 (45/194)	22.3 (21/94)	77.7 (73/94)
	Secondary	53.7 (73/136)	19.1 (26/136)	78.7 (107/136)
	Advanced level	61.5 (8/13)	15.4 (2/13)	84.6 (11/13)
	Certificate	94.7 (18/19)	0 (0/19)	78.9 (15/19)
	Diploma	69.2 (9/13)	23.1 (3/13)	100 (13/13)
	Degree	100 (9/9)	11.1 (1/9)	100 (9/9)
	Post-graduate (masters)	66.7 (2/3)	33.3 (1/3)	100 (3/3)
	None	40 (10/25)	8 (2/25)	68 (17/25)
		χ^2=25.247, p=0.001***	χ^2=8.252, p=0.311	χ^2=8.935, p=0.257
Basic farmer training course	yes	69.4 (86/124)	17.7 (22/124)	85.5 (106/124)
	no	46.8 (88/188)	18.1 (34/188)	76.1 (143/188)
		χ^2=15.398, p<0.0001***	χ^2=0.006, p=0.938	χ^2=4.114, p=0.043*
Problems of ticks and tick borne diseases	yes	60.9 (165/271)	19.2 (52/271)	80.4 (218/271)
	no	21.4 (9/42)	9.5 (4/42)	73.8 (31/42)
		χ^2=22.932, p<0.0001***	χ^2=2.312, p=0.128	χ^2=0.984, p=0.321
Knowledge of TBDs	yes	74.4 (163/219)	22.4 (49/219)	84 (184/219)
	no	9.1 (8/88)	8 (7/88)	70.5 (62/88)
		χ^2=108.609, p<0.0001***	χ^2=8.752, p=0.003***	χ^2=7.254, p=0.007**

6.4. Discussion

This study has demonstrated that centralized dipping programs can be useful in collecting information from farmers which is essential in the management and control of livestock diseases. However, the non-participation of some farmers in surveys conducted on dip days can introduce selection bias which may have influenced the survey results. In this study most farmers were following a consistent dipping program subsidized by the government. As noted by Masika et al., (1997), dipping services are a successful government intervention in the communal livestock production system. In cases where farmers had not dipped their animals for more than a month, it was related to the delay of acaricide supply by the Department of Veterinary Services (DVS). Due to inconsistent cash inflows which are also caused by the fact that the farmers are reluctant to pay dipping levies, the government cannot supply acaricides in time, hence tick control might be affected. It was noted that farmers with large herds were having challenges to pay the dipping levies for all their cattle and hence not all of them were dipping. Discussions with communal farmers also highlighted factors such as water availability and distance to the dip tank as hindering total participation in dipping programs. Dip tanks should ideally be located close to a water source but sometimes this is not the case. It is the responsibility of farmers to fill up the tank with water and they do take turns to do this. However, at some dip tanks disagreements arise as to who will fill up the dip tank and such quarrels may result in water not being available at the dip tank. This normally interrupts dipping programs. In addition, some farmers stay more than 10km from a dip tank and they have to walk long distances to dip their animals. The majority of these cattle owners are old people who are reluctant to walk such long distances and hence for such farmers, dipping is also done during school holidays when school going children take over that job. It will be important to further explore the factors that lead to non-participation of farmers in government dipping programs.

It was important to note that communal farmers related ticks with a number of effects on livestock. The most common were heartwater (cowdriosis) followed by mastitis, gall sickness (anaplasmosis), tick bite wounds, red water (babesiosis) and poor body condition. Clinical and post-mortem signs which are synonymous with TBDs such as red urine (babesiosis), circling movements of cattle (cowdriosis), hard dung (anaplasmosis), hair loss (sweating sickness) and swollen lumps on the neck (theileriosis) were

highlighted by farmers as being caused by ticks. This could have been due to the strong significant association between problem of ticks and ability to name disease. Farmers who had highlighted that TBDs were a problem in the region could either name the disease or describe the signs or symptoms. Munyeme et al., (2010) also found a strong correlation between high prevalence and awareness of a disease. However, farmers incorrectly associated mastitis, joint ill and foot rot as TBDs. Despite these anomalies, farmers seemed to be aware of the major TBDs affecting livestock in the area. This corroborates with the findings of de Garine-Wichatitsky et al., (2013) who observed increased farmer knowledge on the diseases affecting livestock.

The southern low-veld part of Zimbabwe is known to be endemic to heartwater and is characterized by the large presence of the vector tick *Amblyomma hebraeum* (Peter et al., 1999). In this study the prevalence of *Amblyomma hebraeum* ticks was very high being found in almost all the dip tanks (Chapter 3). The predilection sites of the *Amblyomma* ticks are the perineum and inguinal regions where the udders of female cows are located. The greatest numbers of *Amblyomma* ticks are usually found on the udder and their bites would leave wounds which become passages of entry for the bacteria that cause mastitis (Moyo and Masika, 2009). This could probably explain why most farmers attributed mastitis to ticks. During focal group discussions farmers highlighted that in some cases calves died due to failure to suckle because of teat damage caused by ticks. These sentiments are in agreement with findings of Hlatshwayo and Mbati (2005); Masika et al. (1997) and Ndhlovu et al. (2009).

The rickettsial parasites that cause anaplasmosis, *Anaplasma marginale* and *Anaplasma centrale,* are biologically transmitted largely by the vectors *R. decoloratus*, *R. evertsi evertsi* and *R. microplus* (Norval et al., 1984). Anaplasmosis can also be transmitted mechanically through biting insects and use of surgical instruments, and transmission by *Hyalomma rufipes* is also possible (Rikhotso et al., 2005). On the other hand, the protozoal parasites that cause babesiosis, *Babesia bovis* and *Babesia bigemina,* are biologically transmitted by the ticks *R. microplus* and *R. decoloratus*, with the latter only transmitting *B. bigemina* (Mason and Norval, 1980). This could explain the higher ranking of anaplasmosis than babesiosis. Theileriosis was only reported to be a problem by farmers close to the wildlife conservancy where buffaloes (*Syncerus caffer*) are in close contact with domestic livestock. The same observations have also been

reported by de Garine-Wichatitsky et al., (2013). In areas far away from the conservancy it was not reported to be a problem by the farmers. This could be due to the low incidence of the vector tick *R. appendiculatus* in the area which is responsible for the transmission of theileriosis.

Sweating sickness and foot rot were other diseases that farmers associated with ticks. Sweating sickness is a tick-borne disease caused by the release of a toxin by the bont-legged tick *H. truncatum* (Mans et al., 2008). Tick collections indicated the presence of *H. truncatum* in the district and as such incidences of sweating sickness would be expected especially in young calves. However, the prevalence of *H. truncatum* appeared to be low in the district, hence the number of times it was mentioned was also low. Farmers could have associated foot rot with ticks since tick species like the *Hyalomma* spp. can occupy the inter-digital clefts and fetlocks of the animals and cause injuries on the hooves of the animal, thereby predisposing them to foot rot and lameness (Apanaskevich and Horak, 2008).

Most farmers practiced other tick control methods apart from the regular dipping conducted through the DVS and hand spraying by the use of a knapsack was the most common method used by the farmers. Observations indicated that tobacco farming was commonly practiced in the area and the knapsacks that the farmers used to spray their tobacco crop were also used to apply acaricides on their cattle. The formamidines were the common acaricide used by farmers and the DVS. Other acaricides are less common largely because they are more expensive. Furthermore, amitraz is generally viewed as an effective acaricide by farmers (Mugambi et al., 2012). It is also important to note that some farmers used traditional methods of controlling ticks. In this study, the most common traditional way of controlling ticks was the use of black soot mixed with chillies, which is referred to as *chin'ai* in the local Shona language. This substance is ground to a fine consistence and 500g of the mixture is diluted in 10 litres of water, shaken thoroughly and applied on the body of the animal using a broom or tree branch with fine leaves. The efficacy of this method has not been proven scientifically but the farmers suggested that it was effective. The use of traditional methods in controlling ticks is frequently practiced by resource poor communal farmers (Hlatshwayo and Mbati, 2005; Mugambi et al., 2012).

This study has also revealed the need to conduct basic farmer training courses since it was noted that this training had a significant effect on the ability to name disease as well as use of other tick control methods. It has been reported by other researchers that the extension system for livestock production in communal areas is very weak (Chatikobo et al., 2013; Mutibvu et al., 2012). Only a small proportion of farmers indicated that they had undergone basic farmer training and the majority of these were older than 40 years. This group of people could have been trained before the economic meltdown which hit the country and has adversely affected extension services. As a consequence, the majority of the people had not received this training, particularly the younger age classes. The farmers who had undergone training were more aware of TBDs and other tick control methods as compared to the farmers who had not undergone training. The importance of training hence cannot be over emphasized (Peter et al., 2005). Lack of farmer training is one of the constraints in developing farmer managed cost effective tick control programs (Masika et al., 1997). Sex had an effect on the knowledge of TBDs and use of other tick control methods. This could be related to the fact that in most cases males are the keepers of large livestock in communal areas and as such they would be aware of the diseases that affect their livestock. Further, more males had undergone basic farmer training as compared to their female counterparts.

Information gathered from the farmers has indicated that the acaricides being used are effective against ticks. This could be attributed to the high frequency of use of these acaricide chemicals (community dipping coupled with handspraying) by the farmers which will lead to few ticks on the animal. No detailed information exists at the moment with regard to acaricide resistance issues on the major tick species parasitizing livestock in Zimbabwe. However, some farmers did complain of acaricide resistance issues. Acaricide resistance is not only a function of the frequency of acaricide use but of a whole lot of management factors which involve misuse (Adakal et al., 2013; Foil et al., 2004). In one of the dip tanks it was observed that several factors could lead to a reduced efficacy of acaricide treatments. For example due to quarrels farmers do not agree on who is to empty the dip tank and as such the dip becomes heavily silted and the acaricide would sediment at the bottom of the dip. This was observed at one of the dip tanks which had not been replenished with water for close to 12 months. Furthermore, when dipping commences a group of 20-30 animals are required to get into the dip and mix the acaricide with water. This procedure was not followed by the dip

attendants and upon interviewing one of the attendants he did not know the volume of the dip as well as the mixing ratios of water and the acaricide. Incorrect concentration of acaricides is one of the prime reasons for tick control failure at communal dip tanks (Jonsson, 1997).

In conclusion, it is the farmers view that ticks and tick borne diseases pose the greatest threat towards the development of the cattle industry in Zimbabwe. As such, farmers actively participate in government initiated tick control programmes and supplement these with their own programmes. One thing that has been noted is the injudicious use of acaricides which ultimately may lead to acaricide resistance. The status of acaricide resistance in ixodid ticks parasitising particularly cattle in Zimbabwe is not known. It will be important to have first a holistic view of the extent of tick distribution in Zimbabwe highlighting the changes and thereafter investigate the issues of acaricide resistance focusing on at least one of the important tick species. The next chapter is going to look at this.

Chapter 7: Genotyping acaricide resistance profiles of *Rhipicephalus microplus* tick populations from communal land areas of Zimbabwe

This Chapter is based on: Sungirai, M., Baron S., Moyo, D.Z., De Clercq, P., Maritz-Olivier, C., Madder, M. 2017. Genotyping acaricide resistance profiles of *R. microplus* tick populations from communal land areas of Zimbabwe. Ticks and Tick Borne Diseases, 9 (1), 2-9.

7.1. Introduction

The deployment of acaricides is the most widely used strategy for the control of ixodid ticks affecting livestock in tropical and sub-tropical countries of the world (Abbas et al., 2014). The greatest challenge facing its implementation success is the accumulation of resistance in ticks to these chemicals (Rosario-Cruz et al., 2009). Subsequently, effective tick management is premised on the ability to detect and periodically carry out surveillance programmes on acaricide resistance (Ghosh et al., 2015). Bioassays such as the Larval Packet Test (LPT) (Stone and Haydock, 1962), Larval Immersion Test (LIT) (Shaw, 1966) and the Adult Immersion Test (AIT) (Drummond et al., 1973) have traditionally been used to detect and monitor the acaricide resistance status of tick populations while the Larval Tarsal Test (LTT) was recently introduced (Lovis et al., 2013). The increased length of time by which results from a bioassay are obtained, the need for live tick specimens and the inability to detect the genotype status of resistant individuals, negatively influences the effectiveness of these techniques. The development of molecular tools heralded by the design of an allele-specific PCR for the diagnosis of pyrethroid resistance in *Rhipicephalus microplus* ticks (Guerrero et al., 2001) has greatly improved acaricide resistance management. Molecular tools offer the distinct advantage that they are quick, do not require live tick specimens and enable the genotyping of the resistance status of tick populations.

Mutations have been identified in the voltage gated sodium channel gene in *R. microplus* which result in insensitivity to pyrethroids (Miller et al., 1999; He et al., 1999; Jonsson et al., 2010a; Morgan et al., 2009). An esterase in a pyrethroid resistant Mexican *R. microplus* strain was isolated and a mutation in the encoding gene was found by Hernandez et al. (2000), the same mutation was found in organophosphate resistant strains by Baffi et al. (2007). Mutations in genes encoding carboxylesterases will increase esterase hydrolytic activity on an acaricide (Jamroz et al, 2000). Chen et al. (2007) identified two mutations in a putative octopamine receptor gene that will result in target site insensitivity to amitraz which was also confirmed by Baron et al. (2015). These mutations have enabled the development of molecular markers (Guerrero et al, 2001; Morgan et al., 2009; Hernandez et al, 2002; Baron et al., 2015) diagnostic for pyrethroid, organophosphate and amitraz resistance.

Ticks are an important constraint to livestock production in (sub)-tropical countries with annual losses estimated at USD$18.7 billion (De Clercq et al.,2012) and resource-poor communal farmers who own approximately 80% of the cattle are most affected (Rushton et al., 2002). These costs are related to their direct effects as blood sucking parasites which result in productivity losses and damage to hides affecting their quality and value on the market (Rajput et al., 2006). Indirect effects are foreseen in their being: vectors of pathogens which affects humans and animals, costs incurred for treating tick-borne diseases and controlling the vectors (de Castro, 1997). In Zimbabwe the most important tick-borne diseases are heartwater, babesiosis, anaplasmosis and theileriosis and these diseases account for 60 percent of livestock mortalities annually (Chapter 6). In Zimbabwe communal farming systems, tick control is based on the use of a plunge dip, where acaricide chemicals are diluted in large volumes of water and cattle will be submerged in the dip wash. Farmers have to bring their cattle for dipping weekly and fortnightly during the rainy and dry season respectively. The central government supplies acaricides to these communal farmers who pay a nominal fee to the Department of Veterinary Services to have their cattle participate in these tick control programmes (Chapter 6). It then becomes important for the governments to be made aware of the status of acaricide resistance so as to take remedial action. It has been observed that such systems are characterised by : absence of acaricide rotation practises, lack of monitoring of acaricide resistance, no training on the judicious use of acaricides and indiscriminate selling of these chemicals without the recommended active ingredients (Mendes et al., 2013). This may increase the selection pressure for acaricide resistance which may be difficult to reverse once it has been established.

Studies on acaricide resistance have been neglected in Africa although interest in the field is growing (Adakal et al., 2013; Adehan et al., 2016; Baron et al., 2015; Robbertse et al., 2016; Wyk et al., 2016). Much work on acaricide resistance has been reported from Central America, South East Asia, the Caribbean and Australia with the focus on *R. microplus* (*Rhipicephalus australis* in Australia) largely due to its undisputed global importance (Abbas et al., 2014). Further, as a one host tick, the life-cycle stages from larvae to adult are spent on the animal and it will be subjective to more selection pressure for resistance as compared to multiple host ticks (Nolan, 1990, Mekonnen et al., 2002). In the regions describe, this tick species developed resistance to the major classes of acaricides in use, although the level of resistance differs between areas

(Lovis et al., 2012). The purpose of this study was to identify single nucleotide polymorphisms in genes associated with resistance to amidines, pyrethroids and organophosphate acaricides so as to determine whether there is selection pressure for the wild type and the mutant alleles which might influence phenotypic levels of resistance as reported in other studies.

7.2. Materials and Methods

7.2.1. Biological material and DNA extraction

Following a nationwide tick survey conducted in Zimbabwe (Chapter 3), 383 *Rhipicephalus microplus* ticks collected at 103/322 communal dipping tanks were used for this study. The identity of *R. microplus* ticks which could not be resolved through morphology was confirmed using the ITS2 PCR-RFLP test (Lempereur et al., 2010). Whole genomic DNA was extracted from *R. microplus* ticks using the QIAamp genomic DNA kit (Qiagen, Hilden , Germany).

7.2.2. PCR conditions for the octopamine/tyramine receptor gene, carboxylesterase genes and voltage-gated sodium channel genes

The PCR assay conditions for the amplification of the octopamine/tyramine receptor gene, carboxylesterase and voltage-gated sodium channel genes were carried out as described by Baron et al. (2015), Hernandez et al. (2002) and Morgan et al. (2009) respectively. An additional PCR assay as described by Stone et al. (2014) was done to amplify a large fragment of the voltage-gated sodium channel gene for subsequent sequencing. Each of the molecular markers were amplified for the 383 tick samples in a programmable thermocycler (Biometra©, Göttingen, Germany). For the carboxylesterase and octopamine / tyramine receptor genes, Restriction Fragment Length Polymorphism (RFLP) was done after amplification as described by Faza et al. (2013) and Baron et al. (2015) respectively. The PCR and restriction digest products were loaded with dye on a 3% (w/v) agarose gel together with a 100 bp DNA super ladder (Thermo Fisher Scientific©, Waltham, Massachusetts, USA) for sizing the fragments. The gel was stained in 0.001% ethidium bromide solution for 30 minutes and the fragments were visualised using the Gel Doc^{TM} XR^{+} gel documentation system (BioRAd©, Hercules, California, USA). Each reaction was run with a non-template control (nuclease free water) and positive controls of *R. microplus* which were resistant and susceptible to pyrethroids as well as amitraz. These were obtained from the Department of Genetics, University of Pretoria, South Africa.

7.2.3. PCR cloning and sequencing

A sub-set of samples showing homozygous (susceptibility and resistance) as well as heterozygous genotype profiles were selected for cloning and subsequent Sanger sequencing. The QIAquick PCR purification kit (QIAGEN, Hilde, Germany) was used to purify the PCR product according to the manufacturer's instructions. The TOPO® TA Cloning Kit (Thermo Fisher Scientific©, Waltham, Massachusetts, USA) was then used to clone the purified PCR products according to manufacturer's instructions. The positive clones were then sent to the VIB Genetic Service Facility at the University of Antwerp for forward and reverse sequencing.

7.2.4. Data Analysis

Frequency counts were done for all the genotypes present for each marker and these were expressed as a proportion of the total number of individual ticks which had positively amplified. This enabled calculation of the overall proportion of the genotypes as well as the proportion at the provincial level. To test for evidence of selection pressure against the acaricides in use, Hardy-Weinberg Equilibrium (HWE) was examined for the markers where all the genotypes were recorded. This was done using the chi-square test by the software R and a package called “HardyWeinberg” (R Development Core Team, 2013). The forward and reverse sequences were edited using the BioEdit software (Hall, 1999). The final edited sequence was aligned with reference samples of the respective genes to check for the presence of mutations using the software ClustalW (Larkin et al., 2007). These were the para-sodium channel gene, Genebank Accession Number: AF134216.2; for the carboxylesterase, the Gonzalez strain Accession Number : AF182283; and for the octopamine/tyramine receptor gene, the Santa Luisa strain Accession Number: EF490688.1; as well as the Gonzalez strain Accession Number: EF490687.1. Maps showing the geographic distribution of genotypes at the communal dipping tanks where *R. microplus* occurred were generated using QGIS (QGIS Development Team, 2013).

7.3. Results

7.3.1. Single Nucleotide Polymorphisms (SNPs) in the octopamine / tyramine receptor gene

A total of 20 SNPs were found in the open reading frame of the G-protein coupled octopamine / tyramine receptor gene (Figure 7-1). Five of these mutations were transversion mutations in nucleotide positions; 22 (A->C), 141(C->G), 171(G->C), 207 (A->C) and 213 (C->A) while the rest were transition mutations (A<->G and C<->T). Eight nucleotide substitutions were similar to those found in the Santa Luisa strain (resistant) and these included the mutations at nucleotide position 22 (A to C) and 65 (T to C) which are associated with resistance to amitraz (Baron et al., 2015; Chen et al., 2007; He et al., 1999). These mutations are at nucleotide positions 157 and 200 in Baron et al. (2015). The SNP at position 39 in Figure 7-1 which has been found to be in linkage disequilibrium with the SNP at position 65 and found in resistant samples (Baron et al., 2015).

The RFLP profiles for the sequenced samples are shown in Figure 7-2. The expected RFLP profiles were 409 bp for homozygous resistant genotype, 186 bp and 223 bp for the homozygous susceptible genotype, and 409 bp, 223 bp and 186 bp for the heterozygous genotype. Six different RFLP profiles consistently occurred (Figure 7-2 and Table 7-1). As a result, 32 samples were sequenced in order to verify for the presence of SNPs at the expected positions. A check was done to ascertain whether other mutations observed were interfering with the restriction enzyme cutting site thus negatively affecting its utility as a molecular marker. This was done by carrying out a virtual RFLP of sequence data using the programme serial cloner (Perez, 2004). None of these mutations were found to be interfering with the restriction enzyme cutting sites. To genotype the RFLP profiles, these were compared with corresponding sequences to check for the resistant published SNPs i.e. at position 22 and 65. Interestingly some more genotypes other than the ones described by Baron et al. (2015) were found at the SNPs. The RFLP profiles and the corresponding SNPs identified and the frequency of occurrence is shown in Table 7-1.

Table 7-1: RFLP profiles, corresponding SNPs detected, their interpretation and frequency in the examined *R. microplus* samples.

RFLP profile	SNPs detected	Interpretation	% occurrence
409bp	CC/CC	Homozygous-resistant	59/367 (16.1%)
409bp, 223bp, 186bp	CC/TT, CC/TC	Heterozygous	35/367 (9.5%)
409bp, 223bp, 220bp, 190bp, 186bp	AC/TC, CC/TC, AC/TT	Heterozygous	70/367 (19.1%)
409bp, 219bp, 190bp	AC/TC, CC/TC	Heterozygous	183/367 (49.9%)
223bp, 186bp	AA/TT	Homozygous-susceptible	1/367 (0.27%)
219bp, 190bp	AA/TT	Homozygous-susceptible	19/367(5.1%)

Figure 7-1: SNPs observed in the open reading frame of the *R. microplus* G-protein coupled Octopamine tyramine receptor gene (sequences from Zimbabwean samples ORG_09 to ORG_85) were compared with the reference Gonzales strain Gene Bank Accession No. EF490687.1 and the Santa Luisa strain Gene Bank Accession No. EF490688.1). SNPs associated with amitraz resistance are indicated at nucleotide positions 22 and 65.

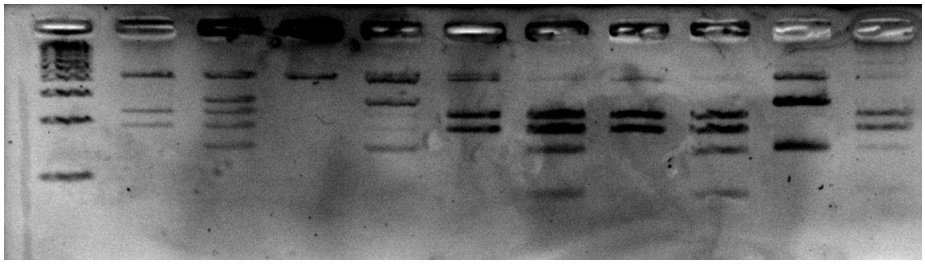


Figure 7-2: RFLP profiles of *R. microplus* samples sent for sequencing (from left to right 1st lane-100bp DNA super ladder, sample identities as in Figure 7-1; ORG_09, ORG_14, ORG_20, ORG_22, ORG_27, ORG_70, ORG_76, ORG_81, ORG_83, ORG_85).

7.3.2. Single Nucleotide Polymorphism in the carboxylesterase gene

Mutations were detected in the homozygous resistant and heterozygous genotypes (Figure 7-3). The published SNP that is associated with resistance is at nucleotide position 1120 (G->A), which is present in the heterozygous ERG_61 and homozygous ERG_65 samples, but absent in the susceptible sample (ERG_001). There were other mutations in the susceptible sample and these occurred in 15 other samples sequenced but they did not interfere with the restriction site, hence they would not affect the utility of the RFLP marker for resistance detection. The corresponding RFLP profiles are shown in Figure 7-4.

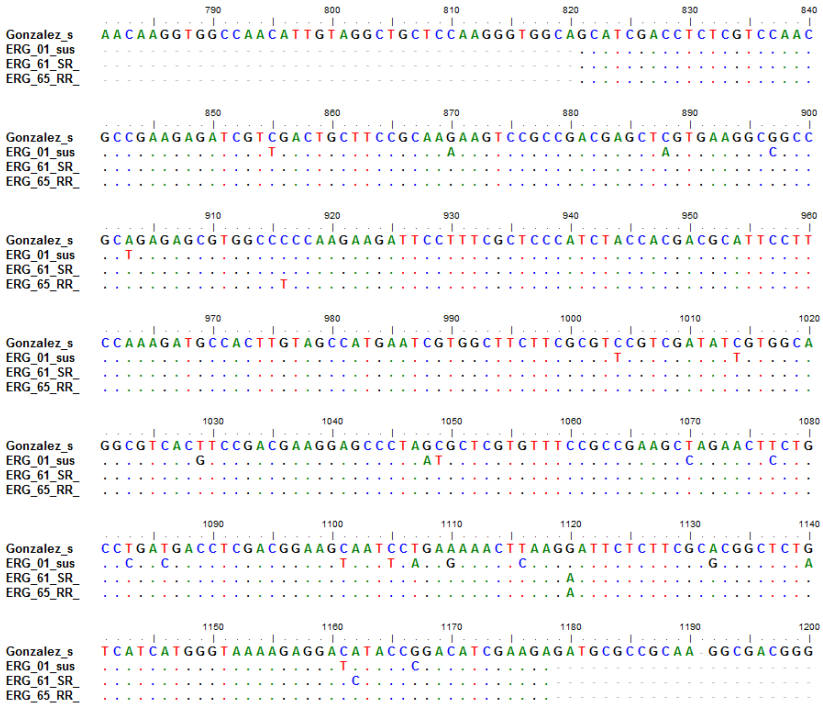


Figure 7-3: Open reading frame of carboxylesterase gene for the 3 samples of *R. microplus* sequenced and compared to the susceptible Gonzalez Strain Gene Bank Accession Number: AF182283

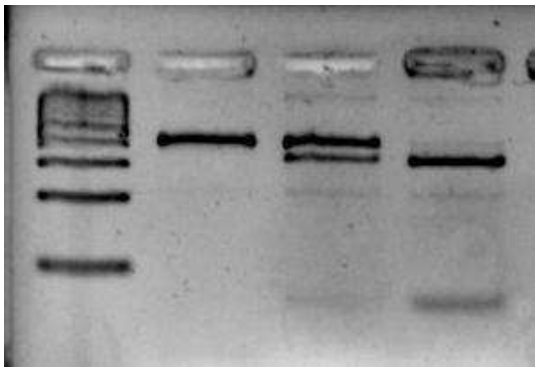


Figure 7-4: RFLP profile for three samples of *R. microplus* whose sequences are shown in Figure 7-3 from left right 100 bp DNA super ladder, ERG_001 (homozygous-susceptible), ERG_061 (heterozygous) and ERG_065 (homozygous-resistant)

7.3.3. Genotype Frequencies and Hardy Weinberg Equilibrium

A total of 383 tick gDNA samples were PCR amplified for the three molecular markers. Not all of the samples were positive for each marker (Table 2). There was a high frequency of amitraz resistance in individual tick samples although a higher proportion of these were heterozygotes (78.5%, n=288/367), with 14.8% being homozygous resistant (n=59/367) and 5.5% (20/367) being susceptible. The proportion of the amitraz resistance-associated alleles in the population was estimated at 55%. For the carboxylesterase marker , 9.8% (n=36/367) were heterozygous whilst 0.54% (n=2/367) were homozygous resistant, the remaining 89.6%, (329/367) were homozygous susceptible. The proportion of the resistant allele for carboxylesterase in the population was estimated at 5.2%. No resistant samples either in the homozygous or heterozygous state were obtained for the L64I mutation. All of the samples were homozygous susceptible (n = 350). HWE revealed selection pressure ($p<0.05$) for amitraz resistance while there was no selection pressure for organophosphates and pyrethroids using the carboxylesterase gene marker ($p>0.05$). Since there were no other genotypes recorded for pyrethroid resistance HWE could not be computed for the C190A mutation.

Table 7-2: Overall frequency of the genotypes

Molecular marker	Genotype			Hardy-Weinberg Equilibrium
	RR	SR	SS	
Octopamine/ Tyramine receptor gene (Amitraz)	59/367, 16.1% (12.3-19.8%)	288/367, 78.5% (74.3-82.7%)	20/367, 5.4% (3.1-7.8%)	χ^2=124.9 p<0.0001
Carboxylesterase (Organophosphate / Pyrethroids)	2/367, 0.52% (0-1.2%)	36/367, 9.4% (6.4-12.3%)	330/367, 85.9% (82.4-89.4%)	χ^2=0.2 p=0.65
Voltage gated sodium channel (Pyrethroids)	0	0	350/350 (100%)	cannot be computed

Complete resistance (RR) for the octopamine/tyramine receptor marker (n=39/103, 37.8%, 95% Confidence Interval (C.I.) estimates 34-53%) and moderate resistance (SR) for the carboxylesterase marker (n= 28/103, 27%, 95% C.I., 31-50%) was recorded at more than a third and close to a third of the dipping tanks respectively, Figure 7-5. The voltage gated sodium channel marker showed susceptible genotypes at all the dipping tanks. Figure 7-6 and Figure 7-7 show the distribution of the genotypes with the "RR" genotype showing complete resistance for amitraz being found in all the provinces where *R. microplus* was collected while for the carboxylesterase marker the genotype was present in two provinces at one dipping tank each. The "SR" genotype at the carboxylesterase marker which shows moderate levels of resistance was found in all the provinces except Matabeleland North.

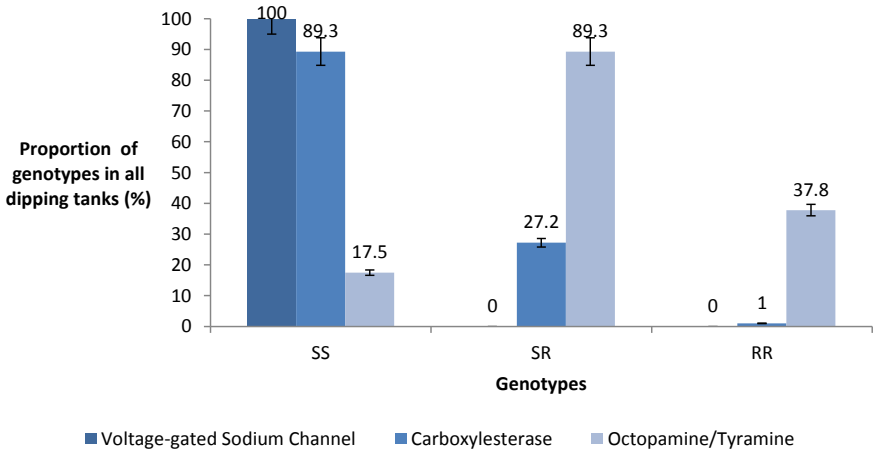


Figure 7-5: Proportion of genotypes for each marker at all the dipping tanks

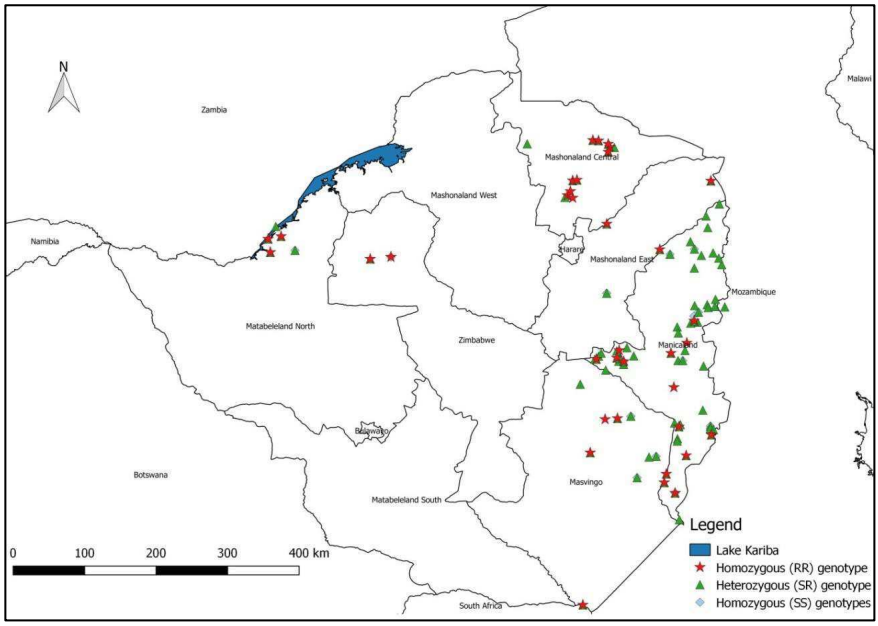


Figure 7-6: Distribution of genotypes at the octopamine/tyramine receptor marker associated with amitraz resistance for *R. microplus* at the communal dipping tanks where the tick species was collected in Zimbabwe

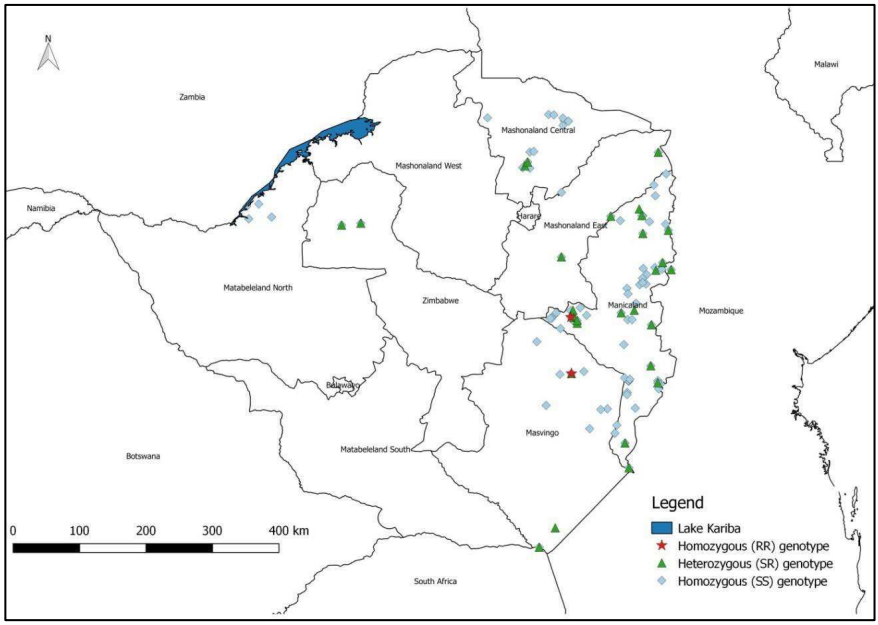


Figure 7-7 : Distribution of genotypes at the carboxylesterase marker associated with pyrethroid and organophosphate resistance in *R. microplus* ticks at communal dipping tanks where the tick species was collected in Zimbabwe

7.4. Discussion

The findings of this study showed that there is high frequency of the resistant allele associated with amitraz resistance in the *R. microplus* population while the mutant allele associated with pyrethroid and organophosphate resistance has a low frequency. These results suggest that the *R. microplus* population in Zimbabwe is undergoing selection pressure towards amitraz resistance and remains susceptible to the pyrethroids and organophosphates in as far as the respective markers studied are concerned.

There was a large proportion of heterozygous genotypes associated with amitraz resistance observed in this study. These results are comparable to a study carried out by Baron et al. (2015) in South Africa where the frequency of the mutant allele was high together with the proportion of heterozygous genotypes. This could be attributed to balancing selection and the inherent fitness costs associated with resistance to amitraz (Corley et al., 2013). Amitraz-resistant strains have been found to lack fitness (Jonsson et al., 2010b) and are recessively inherited (Fragoso-Sanchez et al., 2011), this means that the heterozygous individual will be susceptible to amitraz and the homozygous resistance genotypes will have a selective disadvantage. This leads to a slow rate of fixation of amitraz resistance alleles in the population (Li et al., 2005). Subsequently, balancing selection will act on the population hence a large proportion of heterozygous genotypes. The lack of fitness of amitraz-resistant tick populations could be exploited using an acaricide rotation strategy (Jonsson et al., 2010b). Nonetheless, close to 40% of the dipping tanks showed complete resistance to amitraz, this could be attributed to the high frequency of use of amitraz by communal farmers as reported in Chapter 6. The complete resistance genotypes were found in all the provinces in the country suggesting that the resistance allele is spreading in the country and this could be attributed to the movement of cattle together with *R. microplus* ticks (Chapter 3).

The C190A mutation reported by Morgan et al. (2009) and associated with pyrethroid resistance was not recorded in this study while the carboxylesterase marker also associated with pyrethroid resistance had a low frequency of the mutant allele (five percent) with 10% of the individuals having heterozygous genotypes which were recorded at 27% of the dipping tanks. The carboxylesterase marker has also been associated with organophosphate resistance (Baffi et al., 2007 , Faza et al., 2013). This

low frequency of the resistance genotypes could be attributed to the reduced frequency of use of pyrethroids in communal farms due to their high costs which makes them unaffordable to the resource poor communal farmers (Chapter 6), furthermore this mutation has been associated with low levels of resistance to pyrethroids and OP (Guerrero et al, 2002). The continued use of organophosphates as an acaricide has been discouraged despite them being cheaper than both the pyrethroids and amitraz, due to a number of factors; their toxicity to both the humans and animals, chemical residues are present in meat and milk after slaughter and they can remain undegraded in the environment for close to 30 weeks (De Meneghi et al., 2016). Observations made during tick collection did indicate that the usage of this chemical group of acaricides was very low being applied topically on cattle in the so called hand dressing method which is practised when the tick burden is low and they are a few animals to treat. This explains why the resistant allele was absent in Matabeleland North province where it was observed that communal farmers virtually relied on amitraz for tick control. There is also a fitness cost associated with resistance to pyrethroids and organophosphate whereupon decreased usage of the chemicals might result in the loss of the mutant allele (Stone et al., 2014). Inheritance of resistance in organophosphates and pyrethroids is semi-dominant (Faza et al., 2013), with heterozygous individuals showing moderate levels of resistance. The presence of the heterozygous genotypes by the carboxylesterase marker at nearly 30% of the dipping tanks where *R. microplus* was found is a serious cause for concern.

The absence of a mutation in the sodium channel gene coupled with the presence of one in the carboxylesterase gene presents two contrasting scenarios towards resistance in pyrethroids. Apart from target site insensitivity, metabolic detoxification has been implicated in resistance to pyrethroids and organophosphates especially the increased expression of esterase enzymes which would hydrolyse the acaricide (Rosario-Cruz et al., 2009) and this cannot be detected by molecular assays. It should be noted therefore that the absence of the C190A mutation may not necessarily mean the tick populations are not resistant but that the mechanisms of resistance maybe different for the Zimbabwean tick population. In Mexico resistance mechanisms were found to vary depending on the tick population (Miller et al., 1999) with metabolic detoxification occuring when resistance levels were much lower (Hernandez et al., 2002). The same would apply for organophosphate resistance where apart from the mutation in the

carboxylesterase gene studied here and metabolic detoxification reported in other studies (Li et al., 2005; Baffi et al., 2007), target site insensitivity has been reported in the acetylcholine esterase gene (Temeyer et al., 2010, Ghosh et al., 2015). The results of our study presents an opportunity to further understand resistance mechanisms in *R. microplus* isolates from Zimbabwe.

In conclusion, the continued use of amitraz both by government and supplemental use by farmers indicates selection pressure for resistance. There are other mutations which have been discovered in the octopamine/tyramine receptor gene and it will be important to investigate whether they are associated with resistance. There are still opportunities in Zimbabwe for acaricide rotation using those chemicals which do not appear to be undergoing selection pressure among *R. microplus* at the moment.

Chapter 8: General Discussion, Conclusion and Recommendations

The findings that this study has generated contribute to the growing body of knowledge on the ecology of vector ticks and their management in communal farming systems. The findings are summarised as follows; First, knowledge on the geographic distribution of ixodid ticks in Zimbabwe has been updated; 13 species were collected (*Amblyomma hebraeum, Amblyomma variegatum, Hyalomma rufipes, Hyalomma truncatum, Rhipicephalus appendiculatus, Rhipicephalus compositus, Rhipicephalus decoloratus, Rhipicephalus evertsi evertsi, Rhipicephalus lunulatus, Rhipicephalus microplus, Rhipicephalus near punctatus, Rhipicephalus simus, Rhipicephalus turanicus*), among these eight are of veterinary importance as disease vectors. The range expansion of these tick species continues to change with the evidence of *Rhipicephalus microplus*, *Amblyomma hebraeum* and *Amblyomma variegatum* occupying new areas in Zimbabwe. Secondly, the species *Rhipicephalus microplus* appears to have reached its spatial limit in Zimbabwe unless it manages to adapt to the ecological conditions in newly found areas. This invasive tick has failed to completely displace the indigenous *Rhipicephalus decoloratus* in environments ideal for its proliferation although habitat suitability modelling indicates that this should or would happen. Thirdly, the *R. microplus* tick population in Zimbabwe is panmictic with high levels of gene flow observed between populations leading to a weak population structure. Fourthly, the communal farmers are aware of the dangers posed by ticks and hence consistently participate in community dipping programmes. Fifth, Single-Nucleotide Polymorphisms associated with amidine (amitraz), pyrethroids and organophosphates resistance have been identified in *R. microplus* tick populations from Zimbabwe.

In Chapter 3, an extensive discussion on the factors influencing the distribution of each tick species collected was made. Implications of the expansion in the geographic distribution of *A. hebraeum*, *A. variegatum* and *R. microplus* were also discussed. It is the first time in more than two decades that a nationwide tick survey has been carried out and the results published. Given the observed widespread abundance of *A. hebraeum*, *R. evertsi R. evertsi*, *R. decoloratus* and *R. appendiculatus*, this study indicates that the TBDs, heartwater, anaplasmosis, babesiosis and theilerosis will

continue to be a threat to livestock production in the country. Heartwater as outlined in Chapter 2, is caused by the rickettsial *Erlichia ruminantium* resulting in debilitating losses in non-immune young calves with sheep and goats being the most severely affected. During the study, farmers reported increased cases of heartwater especially in the middle veld where the tick species has now established itself. The diseases, anaplasmosis and babesiosis though widely believed to be endemically stable in most tropical countries where indigenous *Bos Indicus* breeds are kept (Bock et al., 1997) , will hinder the raising of the more productive *Bos Taurus* breeds which are more susceptible to TBDs. The tick species, *Rhipicephalus appendiculatus*, poses a threat, especially with the opening up of wildlife areas for livestock and crop-farming after the land reform (Scoones et al, 2010). This will result in the transmission of *Theileria parva* from buffalo to cattle resulting in the more pathogenic "corridor" disease affecting cattle (Norval et al., 1991a). This was noticeable in Chapter 6, where the disease theilerosis was frequently mentioned by farmers staying adjacent to wildlife conservancies. The presence of the *Hyalomma* species will have implications on the epidemiology of the disease Crimean Congo-Haemorrhagic fever (CCHF) in Zimbabwe which was last investigated three decades ago (Swanepoel et al., 1987). This tick-borne viral zoonosis is increasingly being recognised as a serious cause of human illness with high mortality in Europe, Asia and Africa (Whitehouse, 2004).

Field based studies are essential in understanding the general ecology of a species and form the basis for inferential studies like population genetics and habitat suitability modelling (Lèger et al., 2013). However these can be difficult to conduct especially over long time periods and by a single research team. This study was cross-sectional with no additional visit to the dipping tanks, moreover ticks were collected at different time points. This could have influenced the results obtained especially looking at the low numbers of species like *Rhipicephalus compositus* and the total absence of *Rhipicephalus kochi* which would be expected to occur particularly in the eastern parts of the country (Walker et al, 2000). It is common for such studies to have false negative results (Peter et al., 1998a). The observed intensity of dipping, that is one week intervals during the rainy season or even two-three times a week at the peak of the rainy season could be another factor as well. For *Rhipicephalus kochi* small numbers of adult ticks usually attach to the animal (Walker et al., 2000) and when the frequency of dipping is high, the probability of missing these species during sampling increases.

Berkvens et al. (1998) reported the observation of *R. kochi* towards the end of the rains and beginning of early dry season in Zambia. Much of the tick collections in this study were largely during the rainy season and this could be explain the absence of species like *R. kochi*. This study could be improved by increasing the intensity of sampling during different seasons and also by collecting ticks at the same time point for each dipping tank. Focus should be more on those areas that could not be extensively sampled in this study particularly in the western, southern and northern parts of the country. A quantitative assessment should be made on cattle movements in the country and the reasons behind such movements so as to fully understand the human factors associated with tick spread. The last recorded survey on the sero-prevalence of Babesiosis in the north and eastern parts of Zimbabwe, showed that endemic instability was present in 90% of the herds for *Babesia bigemina* while it was present in 62% of the herds for *Babesia bovis* (Katsande et al., 1996). It would therefore be worthwhile to investigate sero-prevalence of the two pathogens in light of the dynamics in the ecological interactions of their vectors as well as changes in their spatial distribution. More importantly, the epidemiological status of babesiosis and other tick-borne disease have to be investigated as information currently available is either unreliable or outdated. This will help also in analysing the effect of the intensive dipping observed in this study which will have implications on endemic stability (Norval et al., 1983). Continued surveillance of other tick species would be an important management tool (Peter et al., 1998a).

If *R. microplus* has reached its spatial limit, it means that the localisation of control programmes for *Babesia bovis* at foci which favour the proliferation of the vector has to be done. However, as will be explained later, this cannot be entirely achievable due to the sporadic occurrence of the vector tick in unsuitable areas as a result of cattle movements. There might be need to put in place strict and enforceable cattle movements programmes to prevent such occurrences. In this study it could be concluded that *R. microplus* is partially displacing *R. decoloratus* with the two species co-existing at 50% of the dipping tanks where *R. microplus* occurred. As highlighted in Chapter 2 and 3, *R. decoloratus* is expected to be displaced by *R. microplus*. The phenomenon of displacement could be well investigated by looking at tick numbers infesting cattle at these dipping tanks (Nyangiwe et al., 2013a ; Tønnesen et al., 2004) and sampling on alternative hosts in the same area or adjacent areas (Horak et

al.,2009, Sutherst, 1987). This would affirmatively conclude whether displacement is occurring. On another note, this study has generated a fair amount of current spatial distribution of 13 ixodid tick species which could be used to provide habitat suitability maps for other veterinary important tick species; *Amblyomma habreum*, *Amblyomma variegatum* and *Rhipicephalus appendiculatus*. Projections could be made in light of climate change as a risk assessment tool.

Parasites with low vagility will have their genetic structure driven by the mobility of the host species (Blouin et al., 1995). High dispersal rate of host species leads to parasite mixing and panmixia being observed (Kanduma et al., 2015). The spread of ticks into new areas is facilitated by cattle movements (Tønnesen et al., 2004). The panmictic population of *R. microplus* indicates high levels of gene flow between populations which is a consequence of continuous cattle movement. This might have implications in the spread of acaricide resistance alleles (Beesley et al., 2017). In Australia it was noted that amitraz resistance in new areas was more attributed to cattle movements than de novo resistance development (Jonsson and Hope, 2007). In as much as the habitat suitability models indicated the spatial limits of *R. microplus*, the high levels of gene flow observed in the study suggest that there is a high probability of the tick colonising new areas (McCoy, 2008) despite its chances of survival being low. This probably explains the unusual occurrence of this tick species near Lake Kariba. This has implications on vector control programmes, as noted by Tabachnick and Black IV (1995); if there is limited migration between vector populations control programmes will be aimed at focal areas but if vector dispersal is widespread then large scale programmes will be required. As highlighted in Chapter 2, *R. microplus* transmits the more pathogenic *Babesia bovis* infections, colonisation of the tick into new areas will result in mortalities in non-immune cattle which have not been previously exposed to the pathogen (Norval et al., 1983).

The study on the population structure of *R. microplus* is the first to be reported in Zimbabwe and the potential use of eight microsatellite markers for further studies was validated. The markers are polymorphic, neutral, have high PCR efficiency and high levels of average estimates of expected diversity and thus valuable in discriminating between populations (Chevillon et al., 2007). The study could be further enriched by increasing the sampling intensity of individual ticks from each province as has been

proposed by Hale et al. (2012) to have a maximum sample size of 25 individuals per population. In this study, this was initially attempted using 13 microsatellite markers and amplifying 30 samples per provincial population. However from these 150 samples, 87 samples were recovered at eight loci as a result of the frequency of null alleles at the other five loci. Despite their utility, this is one of the drawbacks of using microsatellites (Selkoe and Toonen, 2006). However, the eight loci that were finally selected have a high PCR efficiency of more than 80%. Future studies using these loci should aim at sampling at most 30 individuals per population. The focus of these studies in Zimbabwe should then be on first, the relatedness of acaricide resistant and non-resistant tick populations from the same location compared with resistant populations from other tick populations. This would go a long way in explaining the role cattle movement has in the spread of resistant alleles (Cutullé et al., 2009). Secondly, through population genetics, it may be possible to establish the origins of newly established vector populations, migration patterns as well as the date of invasions, (Chevillon et al., 2013), this would be interesting for the *R. microplus* populations found in different parts of the country and particularly the population near Lake Kariba. Thirdly structuring of population could be influenced by agro-ecological conditions (Kanduma et al., 2015) it would be interesting to investigate how this will influence *R. microplus* population structure. Fourthly, microsatellite markers can be transferred from one species to the other, Van Houtte et al. (2013) isolated and characterised 10 microsatellie loci for *Ixodes arboricola* which could be cross-amplified in their congeners, *Ixodes ricinus*, *I. hexagonus* and *I. frontalis*. This could also be attempted for the microsatellite markers used in our study to see how they perform in *R. decoloratus*. This would help in elucidating the population biology of these species and gain further insights into their ecological interaction (Guzinski et al., 2008) by assessing for instance the impact of control programmes on the effective population size (bottleneck effect) of the two tick species (Delgado-Ratto et al., 2016). The difference in response to acaricide chemicals by these two species has been cited as possibly explaining their interspecific interactions (Chapter 3 and 4). Such a study would provide the necessary evidence. It may be important to also investigate the presence of SNPs in *R. decoloratus* associated with resistance. Recently Vudriko et al. (2017) identified such SNPs for pyrethroids in the VGS of *R. decoloratus* and that could be an important start.

In Chapter 6, an increased participation of farmers in dipping programmes coupled with practise of alternative control methods such as hand spraying and reliance on one type of acaricides was observed. Though well intentioned, this will predispose tick populations to selection pressure for resistance as well as lead to endemic instability (described in Chapter 2) in the advent of a disruption in these programmes. This was once witnessed in Zimbabwe, there had been compulsory consistent dipping of cattle but as the civil war intensified (1978-1980) there was a breakdown in dipping services. This resulted in resurgence of tick populations and approximately a million cattle (33% of the total population) which had not been previously exposed and were non-immune died due to tick-borne diseases (Norval, 1979). In (sub) tropical countries where the majority of cattle breeds are indigenous and well adapted to ticks, the basic aim of tick control is to maintain endemic stability and delay the emergence of acaricide resistance (Mekonnen et al., 2001). As highlighted in Chapter 2, this will rely on a combination of techniques such as strategic treatment, use of resistant cattle breeds, knowledge of tick species as well as their seasonal intensities in different ecological regions (Tatchell, 1992). It might be important to revisit the concept of preserving endemic stability through threshold treatment of indigenous cattle breeds to allow for exposure of cattle to tick-borne diseases so that they gain immunity. It may be necessary for government to revise the policy of weekly and fortnightly interval dipping during the wet and dry season respectively coupled with an acaricide rotation strategy. The use of amitraz has been adopted by the Zimbabwe government since the early 1990s (Norval and Deem, 1994). Although the acaricide may remain effective under heavy use with a general slow emergence of resistance as compared to other acaricides (Jonsson and Hope, 2007), eventually resistance develops as has been reported in Mexico (Benavides et al, 2000, Soberanes et al., 2002). There was a large proportion of farmers using irrational methods of tick control such as hand spraying. This technique is normally practised by farmers with small herds but it is generally viewed as an irrational control practise since its use is usually associated with acaricide wastage and failure for the chemical to reach all cattle body parts (Latif and Walker 2004). In South Africa, a large proportion of resistance was confirmed in farmers using the hand spray as compared to those who used spray races or plunge dips (Spickett and Fivaz, 1992).

As Peter et al. (2005) noted, despite the increased role of the farmers in ectoparasite control, governments should continue to provide support through functional delivery

systems for educational and chemical programmes or other tools required for the vector control. This is so, considering the observation in Chapter 6, that most farmers had not participated in basic farmer training programmes which used to be provided in the past. Education awareness campaigns through veterinary extension services should focus on dipping frequency, use of proper equipment, use of different classes of acaricides and the use of co-formulations which has been adopted in other countries (Jonsson and Matschoss, 1998; Vudriko et al., 2016). Independent observations and general discussions conducted during dipping sessions, indicated that factors such as water availability, distance to the dipping tank, failure to observe standard dipping procedures and other operational factors would influence the success of the dipping programme (Chapter 6). It will be important to carry out empirical studies to establish the extent to which these factors are important for successful tick control programmes. Dipping procedures such as acaricide mixing, dipping tank replenishment, frequency of dipping, should be empirically evaluated as potential risk factors of acaricide resistance.

The identification of SNPs that has been associated with amitraz resistance (Chapter 7) suggests that the *R. microplus* population is undergoing selection pressure for resistance towards this acaricide. This is so, taking into account the high frequency of use of the acaricides by farmers observed in this study (Chapter 3). Amitraz resistance is recessively inherited and is a polygenic trait and as such the emergence of resistance is expected to be slow (Kemp et al., 199, Li et al, 2005). Due to the high fitness costs associated with resistant alleles, resistance will not be maintained in the absence of selection pressure (Foil et al, 2004). This should be exploited by creating acaricide rotation strategies especially taking into account that complete resistant genotypes were observed in approximately 40% of the communal dip tanks. Jonsson et al. (2010b) using the LPT showed that the degree of resistance fluctuated at different times depending on the selection pressure, increasing during the rainy season (high frequency of amitraz use due to high tick load) and decreasing during the dry season (low frequency of amitraz use as a result of low tick load).

This does not hold for selection pressure towards pyrethroids or OPs since for the former, the SNPs which have been associated with high levels of resistance were not identified (Chapter 7). The same applies for organophosphates, where target site insensitivity in the AChe has been suggested as the main mechanism of resistance (Li

et al., 2003; Miller et al., 1999; Temeyer et al., 2010). Metabolic detoxification appears to play a lesser role (Guerrero et al., 2012). The study would have contributed more by focusing on the insensitivity of AChe to OPs as has been described by Temeyer et al. (2010) and more recently by Ghosh et al. (2015). However, the presence of the SNPs at the Cae gene and the absence of SNPs at the VGS gives impetus to explore the resistance mechanisms of Zimbabwean isolates further. The identification of SNPs associated with resistance to amitraz, organophosphate and pyrethroids though very useful would not give a complete picture of resistance since no toxicological bioassays were perfomed. It should be noted that a negative result on PCR amplification for detecting resistant alleles does not mean the tick population is not resistant to the acaricide in question (Jonsson and Hope, 2007). The development of molecular assays may not eliminate the need for toxicological bioassays (Willadsen, 2006). Bioassays would give a strong affirmation of the presence of resistance especially in light of the different resistance mechanisms reported for these acaricides (Guerrero et al, 2012). It will be important that bioassays as well as synergistic studies be done to correlate the SNPs and the phenotypic levels of resistance as well as understand the other resistance mechanisms working in the populations.

Overall, this study has generated information on tick distribution, habitat suitability of two important tick species and the population structure of *R. microplus* which may have important implications on the epidemiology of tick-borne diseases affecting cattle in Zimbabwe. In addition, the management aspects of ticks and tick borne diseases investigated as well as the identification of SNPs associated with acaricide resistance, strongly contributes towards coming up with effective and sustainable vector control strategies.

Summary

Ixodid ticks are responsible for at least 60% of livestock mortalities in Zimbabwe as a result of the diseases that they transmit. They also cause direct and indirect losses in livestock production through their blood sucking activity and as vectors of disease causing pathogens. Costs are also incurred in the control of ticks and treatment of the diseases transmitted. Changes in land use, habitat modifications, climate and human activities have led to shifts in the distribution of ixodid ticks. In Zimbabwe, the government embarked on the land reform programme from the year 2000. The aftermath of the programme resulted in changes in land use patterns and a reconfiguration of the agricultural sector with small scale resettlement farmers now playing a pivotal role. Communal farmers in the so called tribal trust land were moved into areas previously owned by white commercial farmers. This posed a challenge to animal health authorities as animal movement became difficult to regulate. Hence now disease outbreaks are being reported in previously free areas. In addition, the economic meltdown has also had a strain on government veterinary support services such that updated information on disease epidemiology is lacking. In as far as ticks and tick borne diseases are concerned, the last recorded countrywide survey was done in 1996 with a focus on *Amblyomma hebraeum* and *Amblyomma variegatum*. Hence the need to update knowledge on tick distribution. This knowledge will be essential in coming up with control strategies which maybe area specific depending on local tick diversity. Currently the acaricide resistance status of the chemicals in use is not known. This research was carried out to fill these gaps and also to investigate the behaviour of invasive ticks using *Rhipicephalus microplus* as an example looking at adaptability to local conditions, competition with indigenous tick species and acaricide resistance. The involvement of farmers in tick management was also investigated.

The results of the tick survey showed a high diversity and abundance of ixodid ticks parasitising cattle in Zimbabwe. *Amblyomma hebraeum*, *Rhipicephalus evertsi evertsi*, *Rhipicephalus appendiculatus*, *Hyalomma rufipes* and *Rhipicephalus decoloratus* were the widely distributed tick species. *Amblyomma hebraeum* has established itself in the middle Highveld with expansions in the western parts of the country, a predominantly *Amblyomma variegatum* area. In this western part no single *A. variegatum* was collected

suggesting a competitive advantage of *A. hebraeum* largely due to a diversity of alternative wildlife species which serve as hosts for the former. *Amblyomma hebraeum* still has a northern limit where *A. variegatum* appears to have a competitive advantage, but the two tick species co-exist in the Kwekwe area which is part of the middle veld. This would be viewed as a hybrid zone although no hybrids between the two species were recorded. This zone would apparently prevent the spread of either species northwards for *A. hebraeum* and southwards for *A. variegatum*. *Amblyomma variegatum* has spread in the north eastern Highveld an area characterised by above average rainfall and no intense dry season. Subsequently there has been an increase in the incidences of dermatophilosis in the area as reported by the farmers. One revolutionary finding in this study was the recording of *R. microplus* in an arid area in the Zambezi Valley in Binga district. This area does not possess a suitable climate for the survival of this tick, something which was validated by the habitat suitability models. The tick *R. microplus* was also collected in Bikita, a district in Masvingo province suggesting interior spread and the collections in Mazowe and Mt Darwin in Mashonaland Central suggested that the tick species had established there considering that the tick has been previously recorded.

The relationship between *R. microplus* and *R. decoloratus* was explored using climatic variables, altitude and the NDVI. The average environmental requirements of temperature and rainfall are the same for the two species, altitude has the greatest influence on the occurrence of the two species. *Rhipicephalus microplus* hasn't entirely displaced *R. decoloratus* in favourable areas and this has been attributed to unregulated cattle movement between areas suitable for both species, episodic droughts which lead to temporary disappearance of *R. microplus* and the presence of wildlife adjacent to cattle areas especially after the land reform programme. These lead to spill over of *R. decoloratus* from wildlife to cattle when they share the same grazing areas. The ecology of these two tick species in Zimbabwe is different from what is observed in other countries, although from habitat suitability maps, *R. decoloratus* is expected to be displaced by *R. microplus* in areas which favour survival of the latter.

Eight microsatellite loci were used in order to investigate whether *R. microplus* collections are genetically differentiated as a means of local adaptation. These microsatellite loci were found to be highly polymorphic, very efficient and not in linkage

disequilibrium thus providing a starting point for the population study of *R. microplus* in Zimbabwe. Genetic diversity was high (>0.7) in all the populations tested. This suggested high levels of gene flow supporting the hypothesis of unregulated cattle movement between provinces. Genetic differentiation between populations was low (0-0.08) although significant. The *R. microplus* population in Zimbabwe is currently structured in such a way that it is admixed with most of the populations having the same genetic background. However, the population in Matabeleland North has a different genetic background as compared with the others and this is attributed to founder effects. This was as well seen in the finding that the same population had the lowest genetic diversity. This could suggest local adaptation but there is need to investigate this matter further.

Rhipicephalus microplus ticks were screened for resistance to amitraz, pyrethroid and organophosphate acaricides using molecular tools. The results showed selection pressure only for amitraz with more than 80% of tick samples screened having a resistance allele, with approximately 38% of the dipping tanks reporting homozygous resistance to amitraz. The carboxylesterase marker associated with both organophosphate and pyrethroids resistance showed a low proportion of heterozygous individuals (9% of tick samples screened), although these were fairly highly distributed amongst the dipping tanks (27%). There were no resistance alleles detected for pyrethroid resistance in all the 3 markers located in the voltage gated sodium channel gene. This suggests a different mechanism of resistance for *R. microplus* tick populations from Zimbabwe. The sequence results for the octopamine / tyramine receptor gene suggested that there could be other mutations which might play a role in amitraz resistance and this should be investigated further. The results of acaricide resistance screening dovetail with the questionnaire survey which showed that amitraz is the most common acaricide used by communal farmers and government. This would mean that resistance to amitraz is expected to be higher as compared to other acaricides and hence opportunities for rotation are there which would delay full blown resistance.

The questionnaire survey showed that ticks and tick borne disease pose a threat to livestock production in the tropics with more than 80% of respondents reporting problems associated with ticks and tick borne diseases. Heartwater caused by the

rickettsial *Erlichia ruminantium* and transmitted by the tick vectors *Amblyomma hebraeum* and *Amblyomma variegatum* is the most common tick borne disease, followed by anaplasmosis and babesiosis. The majority of the farmers (67%) were able to describe these diseases with clinical or post mortem signs. This also showed that farmers can be a wealthy source of information to understand the epidemiology of diseases. The challenges faced by farmers in tick management in communal areas involve inconsistent supply of acaricides, unaffordable dipping fees, lack of water and lack of knowledge on dipping procedures. Communal farmers will remain an integral part of not only controlling ticks and tick borne diseases but other livestock diseases as well.

A sustainable solution towards the control and management of ticks and tick borne diseases will involve a holistic approach which takes into account social issues to understand farmers' role in the management and control of vectors, ecological factors to have knowledge on the spatial distribution, range limits and variability of tick populations as well as the adoption of novel technologies in acaricide diagnosis.

Samenvatting

Harde teken zijn verantwoordelijk voor ten minste 60% van de vee sterfte in Zimbabwe als gevolg van de ziekten die ze overbrengen. Zij veroorzaken daarenboven directe en indirecte verliezen in de veeteelt omwille van de pathogenen die ze overdragen en ten gevolge van het bloedzuigen. Tenslotte zijn er de kosten, die verbonden zijn aan de controle van de bosluizen en de behandeling van de overgedragen ziekten. Veranderingen in landgebruik, habitat- en klimaatwijzigingen en menselijke activiteiten hebben geleid tot verschuivingen in de distributie van teken. In Zimbabwe heeft de regering vanaf 2000 een landhervormingsprogramma ingevoerd. De nasleep van dit programma leidde tot drastische veranderingen in landgebruik en het hertekenen van de landbouwsector met een nadrukkelijke rol voor kleinschalige, zogenaamde "hervestigingsboeren". Zij werden verplaatst vanuit de traditionele stamgebieden naar zones die tot dusver in bezit waren van blanke commerciële boeren. Dit leidde onmiddellijk tot een zware uitdaging voor de diergezondheidsautoriteiten, omdat de noodzakelijke bewegingen van dieren moeilijk te controleren waren. Daarom werden nu ziekte-uitbraken gerapporteerd in vroeger vrije gebieden. Daarnaast heeft de economische ineenstorting van het land ook invloed gehad op de diergeneeskundige ondersteunende diensten van de overheid, zodat accurate informatie over de epidemiologie van deze uitbraken ontbreekt. De laatste nationale survey naar door teken overgedragen ziekten werd uitgevoerd in 1996, met als focus *Amblyomma hebraeum* en *Amblyomma variegatum*. Vandaar de noodzaak informatie te verzamelen betreffende voorkomen en verdeling van teken. Deze kennis is van essentieel belang om te komen tot specifieke controlestrategieën, aangepast aan de lokale teken populatie. Momenteel is de status van resistentie tegenover de gebruikte acariciden evenmin bekend. Het huidige onderzoek werd uitgevoerd om deze leemtes in te vullen en ook het gedrag van invasieve vectoren te onderzoeken met *Rhipicephalus microplus* als voorbeeld. Hierbij werd gekeken naar aanpassingsvermogen aan lokale omstandigheden, concurrentie met inheemse tekensoorten en acaricide resistentie. Ook de betrokkenheid van boeren bij teken controle werd bekenen.

De resultaten van de enquête toonden een grote diversiteit aan betreffende ixodide tekensoorten op het vee in Zimbabwe: *Amblyomma hebraeum, Rhipicephalus e. evertsi,*

Rhipicephalus appendiculatus, Hyalomma rufipes en *Rhipicephalus decoloratus* waren de meest voorkomende. *Amblyomma hebraeum* heeft zich gevestigd in het midden "Highveld" met uitbreidingen in de westelijke delen van het land, een voormalig *Amblyomma variegatum* gebied. In dit westelijke deel werd geen enkel *A. variegatum* specimen meer verzameld wat het concurrentieel overwicht van *A. hebraeum* aantoont, waarschijnlijk te verklaren door de grote verscheidenheid van aanwezige alternatieve wildsoorten die als gastheer voor deze soort kunnen dienen. *Amblyomma hebraeum* is nog steeds ingeperkt door een noordelijke limiet waar *A. variegatum* een concurrentievoordeel lijkt te hebben, maar de twee soorten komen samen voor in het Kwekwe gebied, dat deel uitmaakt van het middenveld. Dit zou worden gezien als een mogelijke hybridisatie zone, hoewel er geen hybriden tussen de twee soorten werden waargenomen. Deze zone is blijkbaar een bufferzone, die de verspreiding noordwaarts voor *A. hebraeum* en zuidwaarts voor *A. variegatum* voorkomt. *Amblyomma variegatum* heeft zich verspreid in het noordoostelijke Highveld, een gebied dat wordt gekenmerkt door een bovengemiddelde neerslag en de afwezigheid van intense droogte. Een toename van dermatofilose incidentie in dit gebied wordt gerapporteerd door de boeren. Een interessante bevinding in de studie betreft de waarneming van *R. microplus* in het semi-aride gebied van Binga district in de Zambezi vallei: dit gebied beschikt niet over het geschikte klimaat voor de overleving van de soort, wat gevalideerd werd door de bestaande computermodellen die de geschiktheid van habitat testen. *Rhipicephalus microplus* werd ook gevonden in Bikita, een district in Masvingo provincie, wat een interne verspreiding van de soort doet vermoeden en de collecties in Mazowe en Mt Darwin in Mashonaland Central suggereren dat de tekensoort zich daar definitief gevestigd heeft, gezien het feit dat ze daar eerder is waargenomen.

Het voorkomen van respectievelijk *R. microplus* en *R. decoloratus* werd onderzocht aan de hand van klimaatvariabelen, hoogteligging en de NDVI. De gemiddelde temperatuur en neerslag vereisten zijn dezelfde voor de twee soorten en de hoogteligging heeft blijkbaar de grootste invloed op de verspreiding van de twee soorten. *Rhipicephalus microplus* heeft *R. decoloratus* in streken met geschikt klimaat voor beide soorten niet volledig vervangen en dit is mogelijk te verklaren door ongecontroleerde verplaatsen van vee tussen deze gebieden, periodes van droogte die een tijdelijk verdwijnen van *R. microplus* tot gevolg hebben alsmede de aanwezigheid van wild in de buurt van de graaslanden van het vee, en dit na het landhervormingsprogramma, wat leidt tot

overdracht van *R. decoloratus* van wildvee naar rundren waar zij dezelfde weilanden delen. De ecologie van deze twee teken in Zimbabwe is verschillend van waarnemingen in andere landen, aangezien verwacht werd dat *R. decoloratus* door *R. microplus* zou vervangen zijn in zones die de overleving van deze laatste toelaten.

Acht microsatelliet loci werden aangewend om te onderzoeken of *R. microplus* genetisch gedifferentieerd is omwille van lokale aanpassing. Deze microsatelliet loci bleken zeer polymorf te zijn, met hoog scheidingsvermogen en zonder linkage onevenwicht, waardoor ze een uitgangspunt vormen voor de populatie studie van *R. microplus* in Zimbabwe. De genetische diversiteit was hoog (> 0,7) bij alle geteste populaties en er was geen verschil tussen de populaties in termen van diversiteit. Dit veronderstelt hoge niveaus van genenstromen wat de hypothese van aanzienlijke vee migraties tussen de provincies ondersteunt. De genetische differentiatie was laag (0-0,08), hoewel ze significant verschillend was op populatieniveau. De *R. microplus* populatie in Zimbabwe is op dit moment zo gestructureerd er een hoge graad van vermenging optreedt tussen populaties met dezelfde genetische achtergrond. De populatie in Matabeleland Noord heeft echter een verschillende genetische achtergrond vergeleken met de andere en dit wordt toegeschreven aan genetische drift die tot “stichter” effecten leidt. Dit werd bevestigd door de bevinding dat deze populatie de laagste genetische diversiteit had. Dit zou lokale aanpassing kunnen voorstellen, maar deze vraag dient verder onderzocht te worden.

Rhipicephalus microplus werd gescreend op resistentie tegen amitraz, pyrethroide en organofosfaat acariciden met behulp van moleculaire testen. De resultaten toonden selectiedruk aan alleen voor amitraz: bij meer dan 80% van de geteste monsters werd een resistentie-allel gedetecteerd, waarbij 40% van de diptanks een volledige resistentie tegen amitraz meldden. Organofosfaten vertoonden een laag percentage van heterozygote individuen (9% van de stalen), hoewel deze vrij homogeen verdeeld waren over de diptanks (27%). Er waren geen resistentie allelen gedetecteerd voor pyrethroide resistentie in alle 3 markers in het VGNC gen. De sequentieresultaten voor het octopamine/tyramine receptor gen suggereren dat er andere mutaties kunnen zijn die een rol in amitraz resistentie spelen en dit moet verder worden onderzocht. De resultaten van het acaricide-resistentie onderzoek zijn in overeenstemming met de antwoorden verkregen uit de vragenlijst, die aantonen dat amitraz het meest gebruikte

acaricide is bij veehouders en overheden. Dit houdt in dat resistentie tegen amitraz hoger is in vergelijking met andere acariciden. Hierdoor bestaan er mogelijkheden voor rotatie, die de ontwikkeling van volledige weerstand kan afremmen.

Uit de enquête bleek dat teken en door teken overgedragen ziektes een bedreiging vormen voor de veeteelt in de tropen: meer dan 80% van de respondenten vermelden dat ze problemen ondervinden met teken en de door hen overgedragen ziekten. Heartwater, veroorzaakt door het *Erlichia ruminantium* en doorgedragen door de teken vectoren *A. hebraeum* en *A. variegatum* is de meest voorkomende onder hen, gevolgd door anaplasmose en babesiose. De meerderheid van de boeren (67%) waren in staat om deze ziekten te beschrijven met klinische symptomes of post-mortem bevindingen. Het bleek ook dat boeren een belangrijke bron van informatie kunnen zijn om de epidemiologie van ziekten beter te begrijpen. De uitdagingen, die de boeren op het gebied van tekencontrole in de traditionele veehouderij ondervinden, omvatten onregelmatige levering van acariciden, hoge diptank kosten, gebrek aan water en gebrek aan kennis over diptank procedures. Traditionele boeren blijven een integraal onderdeel niet alleen van het beheer van teken, maar ook andere veeziekten. Deze studie heeft aangetoond dat een blijvende oplossing voor de controle en het beheer van teken en door teken overgedragen ziekten een holistische aanpak moet omvatten die rekening houdt met sociale vraagstukken, ecologische factoren en het aanwenden van nieuwe technologie in acaricide resistentie diagnose.

References

Abbas, R.Z., Zaman, M.A., Colwell, D.D., Gilleard, J., Iqbal, Z., 2014. Acaricide resistance in cattle ticks and approaches to its management: the state of play. Veterinary Parasitology 203, 6-20.

Adakal, H., Stachurski, F., Chevillon, C., 2013. Tick control practices in Burkina Faso and acaricide resistance survey in *Rhipicephalus (Boophilus) geigyi* (Acari: Ixodidae). Experimental and Applied Acarology 59, 483-491.

Adehan, S.B., Biguezoton, A., Adakal, H., Assogba, M.N., Zoungrana, S., Gbaguidi, A.M., Tonouhewa, A., Kandè, S., Achi, L., Kagone, H., 2016. Acaricide resistance of *Rhipicephalus microplus* ticks in Benin. African Journal of Agricultural Research 11, 1199-1208.

Angus, B.M., 1996. The history of the cattle tick *Boophilus microplus* in Australia and achievements in its control. International Journal for Parasitology 26, 1341-1355.

Apanaskevich, D.A., Horak, I.G., 2008. The genus *Hyalomma*. VI. Systematics of *H. (Euhyalomma) truncatum* and the closely related species, *H. (E.) albiparmatum* and *H. (E.) nitidum* (Acari: Ixodidae). Experimental and Applied Acarology 44, 115-136.

Araya-Anchetta, A., Busch, J.D., Scoles, G.A., Wagner, D.M., 2015. Thirty years of tick population genetics: A comprehensive review. Infection, Genetics and Evolution 29, 164-179.

Baffi, M.A., de Souza, G.R.L., Vieira, C.U., de Sousa, C.S., Gourlart, L.R., Bonetti, A.M., 2007. Identification of point mutations in a putative carboxylesterase and their association with acaricide resistance in *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). Veterinary Parasitology 148, 301-309.

Balloux, F., Lugon, M.N., 2002. The estimation of population differentiation using microsatellite markers. Molecular ecology 11, 155-165.

Baker, J.A.F., Jordaan, J.O. and Robertson, W.D., 1981. comparison of the resistance spectra to Ixodicides of *Boophilus decoloratus* (Koch) and *Boophilus microplus* (Canestrini) in the Republic of South Africa and Transkei. In Tick biology and control: proceedings of an International Conference January 27-29, 1981/edited by GB Whitehead and JD Gibson. Grahamstown, South Africa: Tick Research Unit, Rhodes University,[1981].

Baron, S., van der Merwe, N.A., Madder, M., Maritz-Olivier, C., 2015. SNP Analysis Infers that Recombination Is Involved in the Evolution of Amitraz Resistance in *Rhipicephalus microplus*. PLoS One 10, e0131341.

Barré, N., Uilenberg, G., 2010. Spread of parasites transported with their hosts: case study of two species of cattle tick. Scientific and Technical Reviews 29, 149-47.

Baxter, G.D., Baker, S.C., 1998. Acetlycholinesterase cDNA of cattle tick, *Boophilus microplus*: characterization and role in organophosphate resistance. Insect Biochem. Mol. Biol. 28, 581–589.

Bazarusanga, T., Geysen, D.F., Vercruysse, J.F., Madder, M., 2007. An update on the ecological distribution of Ixodid ticks infesting cattle in Rwanda: countrywide cross-sectional survey in the wet and the dry season. Experimental and Applied Acarology 43, 279-291.

Beati, L., Keirans, J.E., 2001. Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. Journal of Parasitology 87, 32-48.

Beati, L., Kelly, P.J., Matthewman, L.A., Mason, P.R., Raoult, D., 1995. Prevalence of rickettsia-like organisms and spotted fever group rickettsiae in ticks (Acari: Ixodidae) from Zimbabwe. Journal of Medical Entomology 32, 787-792.

Beesley, N.J., Williams, D.J.L., Paterson, S., Hodgkinson, J., 2017. *Fasciola hepatica* demonstrates high levels of genetic diversity, a lack of population structure and high gene flow: possible implications for drug resistance. International Journal for Parasitology 47, 11-20.

Benavides, E., Rodriguez, J.L., Romero, A., 2000. Isolation and partial characterization of the Montecitos strain of *Boophilus microplus* (Canestrini, 1877) multiresistant to different acaricides. Ann. N. Y. Acad. Sci. 916, 668–671.

Berkvens, D.L., Geysen, D.M., Chaka, G., Madder, M., Brandt, J.R., 1998. A survey of the ixodid ticks parasitising cattle in the Eastern province of Zambia. Medical and Veterinary Entomology 12, 234-240.

Bezuidenhout, J.D., 1985. The epizootiology and control of heartwater and other tick-borne diseases of cattle in South Africa. Onderstepoort Journal of Veterinary Research 52, 211-214.

Bloomquist, J.R., 1993. Toxicology, mode of action and target site-mediated resistance to insecticides acting on chloride channels. Comparative Biochemistry and

Physiology Part C: Pharmacology, Toxicology and Endocrinology, 106(2), pp.301-314.

Blouin, M.S., Yowell, C.A., Courtney, C.H. and Dame, J.B., 1995. Host movement and the genetic structure of populations of parasitic nematodes. Genetics, 141(3), pp.1007-1014.

Bock, R.E., Vos, A.D., Kingston, T.G. and McLellan, D.J., 1997. Effect of breed of cattle on innate resistance to infection with, *Babesia bovis*, *B. bigemina* and *Anaplasma marginale*. Australian veterinary journal, 75(5), pp.337-340.

Booth, T.H., Nix, H.A., Busby, J.R., Hutchinson, M.F., 2014. BIOCLIM: the first species distribution modelling package, its early applications and relevance to most current MAXENT studies. Diversity and Distributions 20, 1-9.

Bournez, L., Cangi, N., Lancelot, R., Pleydell, D.R.J., Stachurski, F., Bouyer, J., Martinez, D., Lefrancois, T., Neves, L., Pradel, J., 2015. Parapatric distribution and sexual competition between two tick species, *Amblyomma variegatum* and *A. hebraeum* (Acari, Ixodidae), in Mozambique. Parasites & Vectors 8, 1-14.

Bruce, D., Mazhowu, W., 1992. Synthetic pyrethroids resistance in *Boophilus decoloratus* : a case report. Zimbabwe Veterinary Journal 26, 127-131.

Burgio, F., Meyer, L., Armstrong, R., 2016. A comparative laboratory trial evaluating the immediate efficacy of fluralaner, afoxolaner, sarolaner and imidacloprid + permethrin against adult *Rhipicephalus sanguineus* (sensu lato) ticks attached to dogs. Parasites & Vectors 9, 626.

Busch, J.D., Stone, N.E., Nottingham, R., Araya-Anchetta, A., Lewis, J., Hochhalter, C., Giles, J.R., Gruendike, J., Freeman, J., Buckmeier, G., Bodine, D., Duhaime, R., Miller, R.J., Davey, R.B., Olafson, P.U., Scoles, G.A., Wagner, D.M., 2014. Widespread movement of invasive cattle fever ticks (*Rhipicephalus microplus*) in southern Texas leads to shared local infestations on cattle and deer. Parasites & Vectors 7, 188-204.

Casida, J.E., 1993. Insecticide action at the GABA-gated chloride channel: Recognition, progress, and prospects. Archives of insect biochemistry and physiology, 22(1-2), pp.13-23.

Catley, A., Osman, J., Mawien, C., Jones, B.A., Leyland, T.J., 2002. Participatory analysis of seasonal incidences of diseases of cattle, disease vectors and rainfall in southern Sudan. Preventive Veterinary Medicine 53, 275-284.

Catley, A.P., Aden, A., 1996. Use of participatory rural appraisal (PRA) tools for investigating tick ecology and tick-borne disease in Somaliland. Tropical Animal Health and Production 28, 91-98.

Catley, A., Alders, R.G., Wood, J.L., 2012. Participatory epidemiology: approaches, methods, experiences. The Veterinary Journal 191, 151-160.

Cole, L.M., Nicholson, R.A. and Casida, J.E., 1993. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. Pesticide Biochemistry and Physiology, 46(1), pp.47-54.

Chatikobo, P., Choga, T., Ncube, C., Mutambara, J., 2013. Participatory diagnosis and prioritization of constraints to cattle production in some smallholder farming areas of Zimbabwe. Preventive Veterinary Medicine 109, 327-333.

Chatikobo, P., Choga, T., Ncube, C., Muzenda-Mutambara, J., 2009. Bovine dermatophilosis, a re-emerging pandemic disease in Zimbabwe. Tropical Animal Health and Production 41, 1289-1297.

Chen, A.C., He, H., Davey, R.B., 2007. Mutations in a putative octopamine receptor gene in amitraz-resistant cattle ticks. Veterinary Parasitology 148, 379-383.

Chen, A.C., He, H., Temeyer, K.B., Jones, S., Green, P. and Barker, S.C., 2009. A survey of *Rhipicephalus microplus* populations for mutations associated with pyrethroid resistance. Journal of economic entomology, 102(1), pp.373-380.

Chenyambuga, S.W., Waiswa, C., Saimo, M., Ngumi, P., Gwakisa, P.S., 2010. Knowledge and perceptions of traditional livestock keepers on tick-borne diseases and sero-prevalence of *Theileria parva* around Lake Victoria Basin. Livestock Research for Rural Development 22.

Chevillon, C., Kofi, B.B., Barre, N., Durand, P., Arnathau, C., de Meeus, T., 2007. Direct and indirect inferences on parasite mating and gene transmission patterns Pangamy in the cattle tick *Rhipicephalus (Boophilus) microplus*. Infection, Genetics and Evolution 7, 298-304.

Chevillon, C., de Garine-Wichatitsky, M., Barre, N., Ducornez, S., De Meeus, T., 2013. Understanding the genetic, demographical and/or ecological processes at play in invasions: lessons from the southern cattle tick *Rhipicephalus microplus* (Acari: Ixodidae). Experimental and Applied Acarology 59, 203-218.

Chigagure, N.N., Baxter, G.D., Barker, S.C., 2000. Microsatellite loci of the cattle tick *Boophilus microplus* (Acari: Ixodidae). Experimental and Applied Acarology 24, 951-956.

Chikerema, S.M., Matope, G.F., Pfukenyi, D.M., 2013. Awareness and attitude toward zoonoses with particular reference to anthrax among cattle owners in selected rural communities of Zimbabwe. Vector. Borne. Zoonotic. Diseases. 13, 243-249.

Chikodzi, D., Murwendo, T. and Simba, F.M., 2013. Climate change and variability in Southeast Zimbabwe: scenarios and societal opportunities. American Journal of Climate Change, 2(03), p.36.

Corley, S.W., Jonsson, N.N., Piper, E.K., Cutullé, C., Stear, M.J., Seddon, J.M., 2013. Mutation in the RmßAOR gene is associated with amitraz resistance in the cattle tick *Rhipicephalus microplus*. Proceedings of the National Academy of Sciences 110, 16772-16777.

Cruickshank, R.H., 2002. Molecular markers for the phylogenetics of mites and ticks. Systematic and Applied Acarology 7, 3-14.

Cumming, G.S., 1999. Host distributions do not limit the species ranges of most African ticks (Acari: Ixodida). Bulletin of Entomological Research 89, 303-327.

Cumming, G.S., 2002. Comparing climate and vegetation as limiting factors for species ranges of African ticks. Ecology 83, 255-268.

Cupp, E.W., 1991. Biology of ticks. Veterinary Clinics of North America: Small Animal Practice 21, 1-26.

Cutullé, C., Jonsson, N.N., Seddon, J., 2009. Population structure of Australian isolates of the cattle tick *Rhipicephalus (Boophilus) microplus*. Veterinary Parasitology 161, 283-291.

Davies, T.G.E., Field, L.M., Usherwood, P.N.R. and Williamson, M.S., 2007. DDT, pyrethrins, pyrethroids and insect sodium channels. Biochemistry and Molecular Biology for Life Sciences, 59(3), pp.151-162.

Davey, R.B., George, J.E. and Snyder, D.E., 2001. Efficacy of a single whole-body spray treatment of spinosad, against *Boophilus microplus* (Acari: Ixodidae) on cattle. Veterinary parasitology, 99(1), pp.41-52.

de Castro, J.J., 1997. Sustainable tick and tickborne disease control in livestock improvement in developing countries. Veterinary Parasitology 71, 77-97.

De Clercq, E.M., Estrada-Pena, A., Adehan, S., Madder, M., Vanwambeke, S.O., 2013. An update on the distribution models for *Rhipicephalus microplus* in West Africa. Geospatial Health 8, 301-308.

De Clercq, E.M., Leta, S., Estrada-Peña, A., Madder, M., Adehan, S., Vanwambeke, S.O., 2015. Species distribution modelling for *Rhipicephalus microplus* (Acari:

Ixodidae) in Benin, West Africa: comparing datasets and modelling algorithms. Preventive Veterinary Medicine 118, 8-21.

De Clercq, E.M., Vanwambeke, S.O., Sungirai, M., Adehan, S., Lokossou, R., Madder, M., 2012. Geographic distribution of the invasive cattle tick *Rhipicephalus microplus*, a country-wide survey in Benin. Experimental and Applied Acarology 58, 441-452.

De Garine-Wichatitsky, M., Miguel, E., Mukamuri, B., Garine-Wichatitsky, E., Wencelius, J., Pfukenyi, D.M., Caron, A., 2013. Coexisting with wildlife in transfrontier conservation areas in Zimbabwe: Cattle owners awareness of disease risks and perceptions of the role played by wildlife. Comparative Immunology, Microbiology and Infectious Diseases 36, 321-332.

De La Fuente, J., Rodríguez, M., Redondo, M., Montero, C., García-García, J., Méndez, L., Serrano, E., Valdés, M., Enriquez, A., Canales, M. and Ramos, E., 1998. Field studies and cost-effectiveness analysis of vaccination with Gavac™ against the cattle tick *Boophilus microplus*. Vaccine, 16(4), pp.366-373.

Delgado-Ratto, C., Gamboa, D., Soto-Calle, V.E., Van den Eede, P., Torres, E., Sánchez-Martínez, L., Contreras-Mancilla, J., Rosanas-Urgell, A., Ferrucci, H.R., Llanos-Cuentas, A. and Erhart, A., 2016. Population genetics of *Plasmodium vivax* in the Peruvian Amazon. PLoS neglected tropical diseases, 10(1), p.e0004376.

De Meeùs, T., Koffi, B.B., Barre, N., Wichatitsky-de Garine, M., Chevillon, C., 2010. Swift sympatric adaptation of a species of cattle tick to a new deer host in New Caledonia. Infection, Genetics and Evolution 10, 976-983.

De Meeùs, T., McCoy, K.D., Chevillon, C., Durand, P., Hurtrez-Bousses, S., Renaud, F., 2007. Population genetics and molecular epidemiology or how to "debusquer la bete". Infection, Genetics and Evolution 7, 308-332.

De Meneghi, D., Stachurski, F. and Adakal, H., 2016. Experiences in tick control by Acaricide in the traditional cattle sector in Zambia and Burkina Faso: possible environmental and public health implications. Frontiers in public health, 4.

De Vos, A.J., Potgieter, F.T., 1994. Bovine babesiosis. In: Infectious Diseases of Livestock. Oxford University Press, Oxford, pp. 278-294.

Deem, S.L., 1998. A review of heartwater and the threat of introduction of *Cowdria ruminantium* and *Amblyomma* spp. ticks to the American mainland. Journal of Zoo and Wildlife Medicine, 109-113.

Delaye, C., Béati, L., Aeschlimann, A., Renaud, F., De Meeùs, T., 1997. Population genetic structure of *Ixodes ricinus* in Switzerland from allozymic data: no evidence of divergence between nearby sites. International Journal for Parasitology 27, 769-773.

Dharmarajan, G., Beasley, J.C. and Rhodes, O.E., 2011. Heterozygote deficiencies in parasite populations: an evaluation of interrelated hypotheses in the raccoon tick, *Ixodes texanus*. Heredity, 106(2), pp.253-260.

Dong, K., 2007. Insect sodium channels and insecticide resistance. Invertebrate Neuroscience 7, 17-30.

Drummond, R.O., Ernst, S.E., Trevino, J.L., Gladney, W.J., Graham, O.H., 1973. *Boophilus annulatus* and *B. microplus*: laboratory tests of insecticides. Journal of Economic Entomology 66, 130-133.

Duncan, I.M., 1992. Tick control on cattle with flumethrin pour-on through a Duncan Applicator. Journal of South African Veterinary Association.

DVS, 2013. Animal Health Situation in Zimbabwe. In. Department of Veterinary Services, Harare, Zimbabwe.

Earl, D.A., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation genetics resources 4, 359-361.

Estrada-Peña, A., 1999. Geostatistics and remote sensing using NOAA-AVHRR satellite imagery as predictive tools in tick distribution and habitat suitability estimations for *Boophilus microplus* (Acari: Ixodidae) in South America. National Oceanographic and Atmosphere Administration-Advanced Very High Resolution Radiometer. Veterinary Parasitology 81, 73-82.

Estrada-Peña, A., Bouattour, A., Camicas, J.L., Guglielmone, A., Horak, I., Jongejan, F., Latif, A., Pegram, R., Walker, A.R., 2006a. The known distribution and ecological preferences of the tick subgenus Boophilus (Acari: Ixodidae) in Africa and Latin America. Experimental and Applied Acarology 38, 219-235.

Estrada-Peña, A., Garcia, Z., Sànchez, H.F., 2006b. The distribution and ecological preferences of *Boophilus microplus* (Acari: Ixodidae) in Mexico. Experimental and Applied Acarology 38, 307-316.

Estrada-Peña, A., Horak, I.G., Petney, T., 2008. Climate changes and suitability for the ticks *Amblyomma hebraeum* and *Amblyomma variegatum* (Ixodidae) in Zimbabwe (1974-1999). Veterinary Parasitology 151, 256-267.

Estrada-Peña, A., 2008. Climate, niche, ticks, and models: what they are and how we should interpret them. Parasitology research 103, 87-95.

Estrada-Peña, A., Alexander, N., Wint, G.W., 2016. Perspectives on modelling the distribution of ticks for large areas: so far so good? Parasites & Vectors 9, 179-186.

Estrada-Peña, A., Estrada-Sánchez, A., Estrada-Sánchez, D., de la Fuente, J., 2013. Assessing the effects of variables and background selection on the capture of the tick climate niche. International journal of health geographics 12, 43.

Estrada-Peña, A., de la Fuente, J., Latapia, T., Ortega, C., 2015. The impact of climate trends on a tick affecting public health: a retrospective modeling approach for *Hyalomma marginatum* (Ixodidae). PLoS One 10, e0125760.

Evans, P.D. and Gee, J.D., 1980. Action of formamidine pesticides on octopamine receptors. Nature, 287(5777), pp.60-62.

Evanno, G., Regnaut, S., Goudet, J.Ã., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular ecology 14, 2611-2620.

FAO, 1984. Acaricide resistance. In: Ticks and tick-borne disease control. A practical field manual Volume 1: 246–299.

Faza, A.P., Pinto, I.S.B., Fonseca, I., Antunes, G.R., de Oliveira Monteiro, C.M.r., Daemon, E., de Souza Muniz, M., Martins, M.F., Furlong, J., de Azevedo Prata, M.r.C., 2013. A new approach to characterization of the resistance of populations of *Rhipicephalus microplus* (Acari: Ixodidae) to organophosphate and pyrethroid in the state of Minas Gerais, Brazil. Experimental parasitology 134, 519-523.

Foil, L.D., Coleman, P., Eisler, M., Fragoso-Sanchez, H., Garcia-Vazquez, Z., Guerrero, F.D., Jonsson, N.N., Langstaff, I.G., Li, A.Y., Machila, N., Miller, R.J., Morton, J., Pruett, J.H., Torr, S., 2004. Factors that influence the prevalence of acaricide resistance and tick-borne diseases. Veterinary Parasitology 125, 163-181.

Fragoso-Sanchez, H., Garcia-Vazquez, Z., Tapia-Perez, G., Ortiz-Najera, A., Rosario-Cruz, R., Rodriguez-Vivas, I., 2011. Response of Mexican *Rhipicephalus (Boophilus) microplus* ticks to selection by amitraz and genetic analysis of attained resistance. Journal of Entomology 8, 218-228.

Fukuto, T.R., 1990. Mechanism of action of organophosphorus and carbamate insecticides. Environmental Health Perspectives, 87, pp.245-254.

Gambiza, J., Nyama, C., 2000. Zimbabwe pasture/forage resource profiles. In. Food and Agriculture Organisation.

Gandon, S., Michalakis, Y., 2002. Local adaptation, evolutionary potential and host-parasite coevolution: interactions between migration, mutation, population size and generation time. Journal of Evolutionary Biology 15, 451-462.

George, J.E., 2000. Present and future technologies for tick control. Annals of New York Academy of Sciences 916, 583-588.

George, J.E., Davey, R.B., Pound, J.M., 2002. Introduced ticks and tick-borne diseases: the threat and approaches to eradication. The Veterinary Clinics Food Animal Practice 18, 401-416.

George, J.E., Pound, J.M., Davey, R.B., 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology 129, S353-S366.

Ghosh, S., Azhahianambi, P. and Yadav, M.P., 2007. Upcoming and future strategies of tick control: a review. Journal of vector borne diseases, 44(2), p.79.

Ghosh, S., Kumar, R., Nagar, G., Kumar, S., Sharma, A.K., Srivastava, A., Kumar, S., Ajith Kumar, K.G., Saravanan, B.C., 2015. Survey of acaricides resistance status of *Rhipiciphalus (Boophilus) microplus* collected from selected places of Bihar, an eastern state of India. Ticks and Tick Borne Diseases 6, 668-675.

Giles, J.R., Peterson, A.T., Busch, J.D., Olafson, P.U., Scoles, G.A., Davey, R.B., Pound, J.M., Kammlah, D.M., Lohmeyer, K.H., Wagner, D.M., 2014. Invasive potential of cattle fever ticks in the southern United States. Parasites & Vectors 7, 189-197.

Gonzalez, J.P., Camicas, J.L., Cornet, J.P., Faye, O., Wilson, M.L., 1992. Sexual and transovarian transmission of Crimean-Congo haemorrhagic fever virus in *Hyalomma truncatum* ticks. Research in virology 143, 23-28.

Gooding, R.H., 1996. Genetic variation in arthropod vectors of disease-causing organisms: obstacles and opportunities. Clinical microbiology reviews 9, 301-320.

Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9. 3). In. http://www2.unil.ch/popgen/softwares/fstat.htm .

Grace, D., Jost, C., MacGregor-Skinner, G., Mariner, J., 2008. Participation of small farmers in animal health programmes. In: 76th General Session of the OIE(Worl Organisation for Animal Health). World Organization for Animal Health, Paris.

Graf, J.F., Gogolewski, R., Leach-Bing, N., Sabatini, G.A., Molento, M.B., Bordin, E.L., Arantes, G.J., 2004. Tick control: an industry point of view. Parasitology 129, S427-S442.

Guerrero, F.D., Jamroz, R.C., Kammlah, D. and Kunz, S.E., 1997. Toxicological and molecular characterization of pyrethroid-resistant horn flies, *Haematobia irritans*: identification of kdr and super-kdr point mutations. Insect biochemistry and molecular biology, 27(8), pp.745-755.

Guerrero, F.D., Davey, R.B., Miller, R.J., 2001. Use of an allele-specific polymerase chain reaction assay to genotype pyrethroid resistant strains of *Boophilus microplus* (Acari: Ixodidae). Journal of Medical Entomology 38, 44-50.

Guerrero, F.D., Li, A.Y. and Hernandez, R., 2002. Molecular diagnosis of pyrethroid resistance in Mexican strains of *Boophilus microplus* (Acari: Ixodidae). Journal of Medical entomology, 39(5), pp.770-776.

Guerrero, F.D. and Pruett, J.H., 2003. Status and future prospects of molecular diagnosis of acaricide resistance in *Boophilus microplus*. Trends Entomol, 3, pp.97-103.

Guerrero, F.D., Lovis, L. and Martins, J.R., 2012. Acaricide resistance mechanisms in *Rhipicephalus (Boophilus) microplus*. Revista Brasileira de Parasitologia Veterinária, 21(1), pp.1-6.

Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., Petney, T.N., Estrada-Peña, A., Horak, I., 2014. The hard ticks of the world. Springer, Dordrecht, Netherlands.

Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., Petney, T.N., Estrada-Peña, A., Horak, I.G., Shao, R., Barker, S.C., 2010. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. Zootaxa 2528, 1-28.

Guisan, A., Thuiller, W., 2005. Predicting species distribution : offering more than simple habitat models. Ecology letters 8, 993-1009.

Guzinski, J., Saint, K.M., Gardner, M.G., Donnellan, S.C. and Bull, C.M., 2008. Permanent genetic resources: Development of microsatellite markers and analysis of their inheritance in the Australian reptile tick, *Bothriocroton hydrosauri*. Molecular ecology resources, 8(2), pp.443-445.

Hahn, M.B., Jarnevich, C.S., Monaghan, A.J., Eisen, R.J., 2016. Modeling the Geographic Distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the Contiguous United States. Journal of Medical Entomology 53, 1176-1191.

Hale, M.L., Burg, T.M. and Steeves, T.E., 2012. Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PloS one, 7(9), p.e45170.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In. [London]: Information Retrieval Ltd., c1979-c2000., pp. 95-98.

He, H., Chen, A.C., Davey, R.B., Ivie, G.W., George, J.E., 1999. Identification of a point mutation in the para-type sodium channel gene from a pyrethroid-resistant cattle tick. Biochemical and Biophysical Research Communications 261, 558-561.

Hendry, D.A. and Rechav, Y., 1981. Acaricidal bacteria infecting laboratory colonies of the tick *Boophilus decoloratus* (Acarina: Ixodidae). Journal of invertebrate pathology, 38(1), pp.149-151.

Hernandez, R., Guerrero, F.D., George, J.E., Wagner, G.G., 2002. Allele frequency and gene expression of a putative carboxylesterase-encoding gene in a pyrethroid resistant strain of the tick B*oophilus microplus*. Insect Biochemistry and Molecular Biology 32, 1009-1016.

Hernandez, R., He, H., Chen, A.C., Waghela, S.D., Ivie, G.W., George, J.E., Wagner, G.G., 2000. Identification of a point mutation in an esterase gene in different populations of the southern cattle tick, *Boophilus microplus*. Insect Biochemistry and Molecular Biology 30, 969-977.

Higa, L.O.S., Garcia, M.V., Barros, J.C., Koller, W.W., Andreotti, R., 2015. Acaricide Resistance Status of the *Rhipicephalus microplus* in Brazil: A Literature Overview. Medicinal Chemistry 5, 326-333.

Hijmans, R.J., Cameron, S., Parra, J., Jones, P., Jarvis, A., Richardson, K., 2005. WorldClim, version 1.3. University of California, Berkeley.

Hijmans, R.J., Elith, J., 2016. Species distribution modeling with R. In. https://cran.r-project.org/web/packages/dismo/vignettes/sdm.pdf.

Hirzel, A.H., Le Lay, G.I., 2008. Habitat suitability modelling and niche theory. Journal of Applied Ecology 45, 1372-1381.

Hirzel, A.H., Le Lay, G.l., Helfer, V.Ã., Randin, C., Guisan, A., 2006. Evaluating the ability of habitat suitability models to predict species presences. Ecological modelling 199, 142-152.

Hlatshwayo, M., Mbati, P.A., 2005. A survey of tick control methods used by resource-poor farmers in the Qwa-Qwa area of the eastern Free State Province, South Africa. Onderstepoort Journal of Veterinary Research 72, p-245.

Horak, I.G., Nyangiwe, N., De, M.C., Neves, L., 2009. Species composition and geographic distribution of ticks infesting cattle, goats and dogs in a temperate and in a subtropical region of south-east Africa. Onderstepoort Journal of Veterinary Research 76, 263-276.

Horak, I.G., Camicas, J.L., Keirans, J.E., 2003. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida): a world list of valid tick names. In: Ticks and Tick-Borne Pathogens. Springer, pp. 27-54.

Hoshino, A.A., Bravo, J.P., Morelli, K.A., Nobile, P.M., 2012. Microsatellites as tools for genetic diversity analysis. In: Caliskan, M. (Ed.), Genetic Diversity in Microorganisms. INTECH Open Access Publisher, Rijeka, Croatia.

Hove, T., Mukandi, R., Bere, M., Horak, I.G., Latif, A.A., 2008. Ixodid ticks infesting domestic goats in communal land areas of Zimbabwe. Journal of South African Veterinary Association 79, 116-120.

IFAD, 2004. A participatory method to understand farmer perceptions of risk so as to improve the delivery, adoption and impact of East Coast fever controlA participatory method to understand farmer perceptions of risk so as to improve the delivery, adoption and impact of East Coast fever control. In. http://www.ifad.org/lrkm/tans/11.htm.

Irvin, A.D., McDermott, J.J., Perry, B.D., 1996. Epidemiology of ticks and tick-borne diseases in eastern, central and southern Africa. Proceedings of a workshop. ILRI (aka ILCA and ILRAD), Nairobi.

James, A.M., Burdett, C.F., McCool MJ, F.A.U., Fox, A.F., Riggs, P., 2015. The geographic distribution and ecological preferences of the American dog tick, *Dermacentor variabilis* (Say), in the U.S.A. Medical and Veterinary Entomology 29, 178-188.

Jamroz, R.C., Guerrero, F.D., Pruett, J.H., Oehler, D.D. and Miller, R.J., 2000. Molecular and biochemical survey of acaricide resistance mechanisms in larvae from

Mexican strains of the southern cattle tick, *Boophilus microplus*. Journal of Insect Physiology, 46(5), pp.685-695.

Jarne, P., Lagoda, P.J., 1996. Microsatellites, from molecules to populations and back. Trends in ecology & evolution 11, 424-429.

Johnson, T.L., Bjork, J.K.H., Neitzel, D.F., Dorr, F.M., Schiffman, E.K. and Eisen, R.J., 2016. Habitat suitability model for the distribution of *Ixodes scapularis* (Acari: Ixodidae) in Minnesota. Journal of medical entomology, 53(3), pp.598-606.

Jongejan, F., Uilenberg, G., 1994. Ticks and control methods. Scientific and Technical Reviews 13, 1201-1226.

Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. Parasitology 129 Suppl, S3-14.

Jonsson, N.N., 1997. Control of cattle ticks (*Boophilus microplus*) on Queensland dairy farms. Australian veterinary journal 75, 802-807.

Jonsson, N.N. and Matschoss, A.L., 1998. Attitudes and practices of Queensland dairy farmers to the control of the cattle tick, *Boophilus microplus*. Australian veterinary journal, 76(11), pp.746-751.

Jonsson, N.N., 2004. Integrated control programs for ticks on cattle: An: Examination of some possible components. FAO animal production and health paper. School of Veterinary Science, University of Queensland, Australia.

Jonsson, N.N., Cutulle, C.F., Corley SW, F.A.U., Seddon, J.M., 2010a. Identification of a mutation in the para-sodium channel gene of the cattle tick *Rhipicephalus microplus* associated with resistance to flumethrin but not to cypermethrin. International Journal of Parasitology 40, 1659-1664.

Jonsson, N.N., Hope, M., 2007. Progress in the epidemiology and diagnosis of amitraz resistance in the cattle tick *Boophilus microplus*. Veterinary Parasitology 146, 193-198.

Jonsson, N.N., Miller, R.J., Kemp, D.H., Knowles, A., Ardila, A.E., Verrall, R.G., Rothwell, J.T., 2010b. Rotation of treatments between spinosad and amitraz for the control of *Rhipicephalus (Boophilus) microplus* populations with amitraz resistance. Veterinary Parasitology 169, 157-164.

Justice, C.O., Vermote, E., Townshend, J.R., Defries, R., Roy, D.P., Hall, D.K., Salomonson, V.V., Privette, J.L., Riggs, G., Strahler, A., 1998. The Moderate Resolution Imaging Spectroradiometer (MODIS): Land remote sensing for global

change research. IEEE Transactions on Geoscience and Remote Sensing 36, 1228-1249.

Kagaruki, L.K., 1991. Tick (Acari: Ixodidae) resistance to organochlorine acaricides in Tanzania. Tropical Pest Management 37, 33-36.

Kanduma, E.G., Mwacharo, J.M., Mwaura, S., Njuguna, J.N., Nzuki, I., Kinyanjui, P.W., Githaka, N., Heyne, H., Hanotte, O., Skilton, R.A., Bishop, R.P., 2015. Multi-locus genotyping reveals absence of genetic structure in field populations of the brown ear tick (*Rhipicephalus appendiculatus*) in Kenya. Ticks and Tick Borne Diseases 7, 26-35.

Katsande, T.C., More, S.J., Bock, R.E., Mabikacheche, L., Molloy, J.B., Ncube, C., 1999. A serological survey of bovine babesiosis in northern and eastern Zimbabwe. Onderstepoort Journal of Veterinary Research 66, 255-263.

Katsande, T.S., Mazhowu Turton, J.A., Munodzana, D., 1996. *Babesia bovis* case reports and the current distribution of *Boophilus microplus* in Zimbabwe. Zimbabwe Veterinary Journal 27, 33-36.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647-1649.

Kemp, D. H., K. R. Gale, A. Nari, and G. A. Sabatini. 1998. Acaricide resistance in the cattle-ticks *Boophilus microplus* and *B. decoloratus*: review of resistance data; standardization of resistance tests and recommendations for the integrated parasite control to delay resistance. CSIRO Tropical Agriculture, Long Pocket Laboratories, Indooroopilly, Australia.

Kemp, D. H., Mckenna, R. V., Thullner, R. & Willadsen, P. (1999). Strategies for tick control in a world of acaricide resistance. Proceedings of the IV Seminario Internacional de Parasitologia Animal, pp. 1–10. Puerto Vallarta, Mexico October 20th–22nd, 1999.

Kim, K.S. and Sappington, T.W., 2013. Microsatellite data analysis for population genetics. Microsatellites: Methods and Protocols, pp.271-295.

Koffi, B.B., Risterucci, A.M., Joulia, D., Durand, P., Barre, N., De Meeùs, T., Chevillon, C., 2006. Characterization of polymorphic microsatellite loci within a young *Boophilus microplus* metapopulation. Molecular ecology notes 6, 502-504.

Koh-Tan, H.C., Strachan, E., Cooper, K., Bell-Sakyi, L. and Jonsson, N.N., 2016. Identification of a novel β-adrenergic octopamine receptor-like gene (βAOR-like) and increased ATP-binding cassette B10 (ABCB10) expression in a Rhipicephalus microplus cell line derived from acaricide-resistant ticks. Parasites & vectors, 9(1), p.425.

Kunz, S.E. and Kemp, D.H., 1994. Insecticides and acaricides: resistance and environmental impact. Revue scientifique et technique (International Office of Epizootics), 13(4), pp.1249-1286.

Lamy, E., van Harten, S., Sales-Baptista, E., Guerra, M.M.M., de Almeida, A.M., 2012. Factors influencing livestock productivity. In: Sejian, V., Naqvi, S.M.K., Ezeji, T., Lakritz, J., Lal, R. (Eds.), Environmental stress and amelioration in livestock production. Springer, London, pp. 19-51.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947-2948.

Latif, A.A., Hove, T., Kanhai, G.K., Masaka, S., Pegram, R.G., 2001. Epidemiological observations of Zimbabwean theileriosis: disease incidence and pathogenicity in susceptible cattle during *Rhipicephalus appendiculatus* nymphal and adult seasonal activity. Onderstepoort Journal of Veterinary Research 68, 187-195.

Latif, A.A. and Walker, A.R., 2004. An introduction to the biology and control of ticks in Africa. ICTTD-2 project, pp.1-29.

Lèger, E., Vourc'h, G., Vial, L., Chevillon, C., McCoy, K.D., 2013. Changing distributions of ticks: causes and consequences. Experimental and Applied Acarology 59, 219-244.

Lempereur, L., Geysen, D., Madder, M., 2010. Development and validation of a PCR-RFLP test to identify African *Rhipicephalus (Boophilus)* ticks. Acta tropica 114, 55-58.

Li, A.Y., Davey, R.B., Miller, R.J. and George, J.E., 2003. Resistance to coumaphos and diazinon in *Boophilus microplus* (Acari: Ixodidae) and evidence for the involvement of an oxidative detoxification mechanism. Journal of medical entomology, 40(4), pp.482-490.

Li, A.Y., Davey, R.B., Miller, R.J. and George, J.E., 2004. Detection and characterization of amitraz resistance in the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae). Journal of medical entomology, 41(2), pp.193-200.

Li, A.Y., Davey, R.B., Miller, R.J., George, J.E., 2005. Mode of inheritance of amitraz resistance in a Brazilian strain of the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae). Experimental and Applied Acarology 37, 183-198.

Lorusso, V., Picozzi, K., de Bronsvoort, B.M., Majekodunmi, A., Dongkum, C., Balak, G., Igweh, A., Welburn, S.C., 2013. Ixodid ticks of traditionally managed cattle in central Nigeria: where *Rhipicephalus (Boophilus) microplus* does not dare (yet?). Parasites & Vectors 6, 171-180.

Lovis, L., Guerrero, F.D., Miller, R.J., Bodine, D.M., Betschart, B., Sager, H., 2012. Distribution patterns of three sodium channel mutations associated with pyrethroid resistance in *Rhipicephalus (Boophilus) microplus* populations from North and South America, South Africa and Australia. International Journal for Parasitology: Drugs and Drug Resistance 1, 230-270.

Lovis, L., Mendes, M.C., Perret, J.L., Martins, J.R., Bouvier, J., Betschart, B., Sager, H., 2013. Use of the Larval Tarsal Test to determine acaricide resistance in *Rhipicephalus (Boophilus) microplus* Brazilian field populations. Veterinary Parasitology 191, 323-331.

Luguru, S.M., Chizyuka, H.G.B., Musisi, F.L., 1987. A survey of resistance to acaricides in cattle tick (Acari: Ixodidae) in three major traditional cattle areas of Zimbabwe. Bulletin of Entomological Research 77, 569-574.

Lynen, G., Zeman, P., Bakuname, C., Di, G.G., Mtui, P., Sanka, P., Jongejan, F., 2008. Shifts in the distributional ranges of *Boophilus* ticks in Tanzania: evidence that a parapatric boundary between *Boophilus microplus* and *B. decoloratus* follows climate gradients. Experimental and Applied Acarology 44, 147-164.

Madder, M., Adehan, S., De Deken, R., Adehan, R., Lokossou, R., 2012. New foci of *Rhipicephalus microplus* in West Africa. Experimental and Applied Acarology 56, 385-390.

Madder, M., Thys, E., Achi, L., Touré, A., De Deken, R., 2011. *Rhipicephalus (Boophilus) microplus*: a most successful invasive tick species in West-Africa. Experimental and Applied Acarology 53, 139-145.

Madder, M., Speybroeck, N., Brandt, J., Tirry, L., Hodek, I., Berkvens, D., 2002. Geographic variation in diapause response of adult *Rhipicephalus appendiculatus* ticks. Experimental and Applied Acarology 27, 209-221.

Madder, M., Thys, E., Geysen, D., Baudoux, C., Horak, I., 2007. *Boophilus microplus* ticks found in West Africa. Experimental and Applied Acarology 43, 233-234.

Madzimure, J., Nyahangare, E.T., Hamudikuwanda, H., Hove, T., Belmain, S.R., Stevenson, P.C. and Mvumi, B.M., 2013. Efficacy of *Strychnos spinosa* (Lam.) and *Solanum incanum* L. aqueous fruit extracts against cattle ticks. Tropical animal health and production, 45(6), pp.1341-1347.

Manel, S., Williams, H.C., Ormerod, S.J., 2001. Evaluating presence-absence models in ecology: the need to account for prevalence. Journal of Applied Ecology 38, 921-931.

Mans, B.J., Gothe, R., Neitz, A.W.H., 2008. Tick toxins: perspectives on paralysis and other forms of toxicoses caused by ticks. In: Bowman, A.S., Nutall, P.A. (Eds.), Ticks: biology, disease and control. Cambridge University Press, Cambridge, pp. 108-126.

Mariner, J.C., Hendrickx, S., Pfeiffer, D.U., Costard, S., Knopf, L., Okuthe, S., Chibeu, D., Parmley, J., Musenero, M., Pisang, C., 2011. Integration of participatory approaches into surveillance systems. Scientific and Technical Reviews 30, 653-659.

Masika, P.J., Sonandi, A., Van Averbeke, W., 1997. Tick control by small-scale cattle farmers in the central Eastern Cape Province, South Africa. Journal of the South African Veterinary Association 68, 45-48.

Mason, C.A., Norval, R.A.I., 1980. The ticks of Zimbabwe. I. The genus *Boophilus*. Zimbabwe Veterinary Journal 11, 36-43.

Mavedzenge, B.Z., Mehenehene, J., Murimbarimba, F., Scoones, I., 2006. Changes in the livestock sector in Zimbabwe following land reform: The case of Masvingo province. In. http://www.ids.ac.uk/.

McCoy, K.D., Boulinier, T., Tirard, C. and Michalakis, Y., 2003. Host-dependent genetic structure of parasite populations: differential dispersal of seabird tick host races. Evolution, 57(2), pp.288-296.

McCoy, K.D., 2008. The population genetic structure of vectors and our understanding of disease epidemiology. Parasite Journal 15, 444-448.

McCoy, K.D., Beis, P., Barbosa, A.s., Cuervo, J.J., Fraser, W.R., González-Solís, J., Jourdain, E., Poisbleau, M., Quillfeldt, P., Lèger, E., 2012. Population genetic structure and colonisation of the western Antarctic Peninsula by the seabird tick Ixodes uriae. Marine Ecology Progress Series 459, 109-120.

McTier, T.L., Chubb, N., Curtis, M.P., Hedges, L., Inskeep, G.A., Knauer, C.S., Menon, S., Mills, B., Pullins, A., Zinser, E., Woods, D.J., Meeus, P., 2016. Discovery of

sarolaner: A novel, orally administered, broad-spectrum, isoxazoline ectoparasiticide for dogs. Veterinary Parasitology 222, 3-11.

Meirmans, P.G., 2009. GenoDive version 2.0 b14. Computer software distributed by the author. Available from: http://www. bentleydrummer. nl/software/software/GenoDive. html.

Mekonnen, S., Bryson, N.R., Fourie, L.J., Peter, R.J., Spickett, A.M., Taylor, R.J., Strydom, T., Horak, I.G., 2002. Acaricide resistance profiles of single- and multi-host ticks from communal and commercial farming areas in the Eastern Cape and North-West Provinces of South Africa. Onderstepoort Journal of Veterinary Research 69, 99-105.

Mekonnen, S., Hussein, I. and Bedane, B., 2001. The distribution of ixodid ticks (Acari: Ixodidae) in central Ethiopia. The Onderstepoort journal of veterinary research, 68(4), p.243.

Mendes, M.C., Duarte FC, Martins, J.R., Klafke, G.M.,Fiorini, L.C., de Barros, A.T., 2013. Characterization of the pyrethroid resistance profile of *Rhipicephalus (Boophilus) microplus* populations from the states of Rio Grande do Sul and Mato Grosso do Sul, Brazil. Revista Brasileira de Parasitologia Veterinária 22, 379-384.

Miller, R.J., Davey, R.B. and George, J.E., 1999. Characterization of pyrethroid resistance and susceptibility to coumaphos in Mexican *Boophilus microplus* (Acari: Ixodidae). Journal of medical entomology, 36(5), pp.533-538.

Miller, R.J., Davey, R.B. and George, J.E., 2002. Modification of the food and agriculture organization larval packet test to measure amitraz-susceptibility against Ixodidae. Journal of medical entomology, 39(4), pp.645-651.

Minjauw, B., Mcleod, A., 2003. Tick Borne Diseases and Poverty: The Impact of ticks and tick borne diseases on the livelihood of small scale and marginal livestock owners in India and eastern and southern Africa. Department For International Development, Edinburgh.

Minjauw, B., Muriuki, H.G., Romney, D., 2002. Development of farmer field school (FFS) methodology for smallholder dairy farmers in Kenya. In. ILRI, Nairobi, pp. 91-103.

Mixson, T.R., Lydy, S.L., Dasch, G., Real, L.A., 2006. Inferring the population structure and demographic history of the tick, *Amblyomma americanum* Linnaeus. Journal of Vector Ecology 31, 181-192.

Morgan, J.A., Corley, S.W., Jackson, L.A., Lew-Tabor, A.E., Moolhuijzen, P.M., Jonsson, N.N., 2009. Identification of a mutation in the para-sodium channel gene of the cattle tick *Rhipicephalus (Boophilus) microplus* associated with resistance to synthetic pyrethroid acaricides. International Journal of Parasitology 39.

Mosalagae, D., Pfukenyi DM FAU - Matope, G., Matope, G., 2011. Milk producers' awareness of milk-borne zoonoses in selected smallholder and commercial dairy farms of Zimbabwe. Tropical Animal Health and Production 43, 733-739.

Moyo, B., Masika, P.J., 2009. Tick control methods used by resource-limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. Tropical Animal Health and Production 41, 517-523.

Mugambi, J.M., Wesonga, F.D., Ndungu, S.G., 2012. Ticks and tick-borne disease control in a pastoral and an agro-pastoral farming systems in Kenya. Livestock Research for Rural Development 24.

Mugisha, A., McLeod, A., Percy, R., Kyewalabye, E., 2005. Strategies, effectiveness and rationale of vector-borne disease control in the pastoralist system of south-western Uganda. Tropical Animal Health and Production 37, 479-489.

Mugisha, A., McLeod, A., Percy, R., Kyewalabye, E., 2008. Socio-economic factors influencing control of vector-borne diseases in the pastoralist system of south western Uganda. Tropical Animal Health and Production 40, 287-297.

Mukhebi, A.W., Chamboko, T., O'Callaghan, C.J., Peter, T.F., Kruska, R.L., Medley, G.F., Mahan, S.M., Perry, B.D., 1999. An assessment of the economic impact of heartwater (*Cowdria ruminantium* infection) and its control in Zimbabwe. Preventive Veterinary Medicine 39, 173-189.

Mullin, C.A. and Scott, J.G. eds., 1992. Molecular mechanisms of insecticide resistance: diversity among insects. American Chemical Society, Washington DC, USA.

Munyeme, M., Muma JB FAU - Munang'andu, H., Munang'andu HM FAU - Kankya, C., Kankya, C.F., Skjerve E FAU - Tryland, M., Tryland, M., 2010. Cattle owners' awareness of bovine tuberculosis in high and low prevalence settings of the wildlife-livestock interface areas in Zambia. BMC Veterinary Research 6.

Murrell, A., Campbell, N.J., Barker, S.C., 2000. Phylogenetic analyses of the rhipicephaline ticks indicate that the genus *Rhipicephalus* is paraphyletic. Molecular Phylogenetics and Evolution 16, 1-7.

Mutibvu, T., Maburutse, B.E., Mbiriri, D.T., Kashangura, M.T., 2012. Constraints and opportunities for increased livestock production in communal areas: A case study of Simbe, Zimbabwe. Livestock Research for Rural Development 24.

Muyobela, J., Nkunika, P.O., Mwase, E.T., 2015. Resistance status of ticks (Acari; Ixodidae) to amitraz and cypermethrin acaricides in Isoka District, Zambia. Tropical Animal Health and Production 47, 1599-1605.

Nauen, R. and Bretschneider, T., 2002. New modes of action of insecticides. Pesticide Outlook, 13(6), pp.241-245.

Navajas, M., Fenton, B., 2000. The application of molecular markers in the study of diversity in acarology: a review. Experimental and Applied Acarology 24, 751-774.

Ndhlovu, D.N. and Masika, P.J., 2013. Ethno-veterinary control of bovine dermatophilosis and ticks in Zhombe, Njelele and Shamrock resettlement in Zimbabwe. Tropical animal health and production, 45(2), pp.525-532.

Ndhlovu, D.N., Makaya, P.V., Penzhorn, B.L., 2009. Tick infestation, and udder and teat damage in selected cattle herds of Matabeleland South, Zimbabwe. Onderstepoort Journal of Veterinary Research 76, 235-248.

Needham, G.R., Teel, P.D., 1991. Off-Host Physiological Ecology of Ixodid Ticks. Annual Review of Entomology 36, 659-681.

Norval, R.A.I., 1979. Tick infestations and tick-borne diseases in Zimbabwe Rhodesia. Journal of the South African Veterinary Association, 50(4), pp.289-292.

Norval, R.A., Fivaz, B.H., Lawrence, J.A., Brown, A.F., 1984. Epidemiology of tick-borne diseases of cattle in Zimbabwe. II. Anaplasmosis. Tropical Animal Health and Production 16, 63-70.

Nolan, J., 1990. Acaricide resistance in single and multi-host ticks and strategies for control. Parassitologia, 32(1), pp.145-153.

Norval, R.A., Fivaz, B.H., Lawrence, J.A., Daillecourt, T., 1983. Epidemiology of tick-borne diseases of cattle in Zimbabwe. I. Babesiosis. Tropical Animal Health and Production 15, 87-94.

Norval, R.A., Perry, B.D., 1990. Introduction, spread and subsequent disappearance of the brown ear-tick, *Rhipicephalus appendiculatus*, from the southern lowveld of Zimbabwe. Experimental and Applied Acarology 9, 103-111.

Norval, R.A., Perry, B.D., Hargreaves, S.K., 1992a. Tick and tick-borne disease control in Zimbabwe: what might the future hold. Zimbabwe Veterinary Journal 23, 1-15.

Norval, R.A., Perry, B.D., Meltzer, M.I., Kruska, R.L., Booth, T.H., 1994. Factors affecting the distributions of the ticks *Amblyomma hebraeum* and *A. variegatum* in Zimbabwe: implications of reduced acaricide usage. Experimental and Applied Acarology 18, 383-407.

Norval, R.A.I., 1982. Ticks of Zimbabwe. IV. The genus *Hyalomma*. Zimbabwe Veterinary Journal 13, 2-10.

Norval, R.A.I., 1983. Ticks of Zimbabwe. VII. The genus *Amblyomma*. Zimbabwe Veterinary Journal 14, 3-6.

Norval, R.A.I., 1985a. The control of ticks and tick borne diseases in Zimbabwe. Zimbabwe Science News 19, 19-20.

Norval, R.A.I., Lawrence, J.A., Young, A.S., Perry, B.D., Dolan, T.T., Scott, J., 1991a. *Theileria parva*: influence of vector, parasite and host relationships on the epidemiology of theileriosis in southern Africa. Parasitology 102, 347-356.

Norval, R.A.I., Meltzer, M.I., Burridge, M.J., 1991b. Distribution, economic importance and control measures for *Cowdria ruminantium*. In: Recent Developments in the Control of Anaplasmosis, Babesiosis and Cowdriosis. Proceedings of a workshop held at ILRAD, Nairobi, Kenya. ILRAD, Nairobi, pp. 13-15.

Norval, R.A.I., Perry, B.D., Kruska, R., Kundert, K., 1991c. The use of climatic data interpolation to estimate the distribution of *Amblyomma variegatum* in Africa. Preventive Veterinary Medicine 11, 365-366.

Norval, R.A.I. and Deem, S.L., 1994. The development and application of models in the planning and implementation of reduced and strategic-minimal tick control strategies in Zimbabwe. Modelling of vector-borne and other parasitic diseases (Perry, BD and Hansen, JW, eds.) ILRAD, Nairobi, Kenya, pp.283-295.

Norval, R.A., Perry, B.D., Young, A.S., 1992b. The epidemiology of theileriosis in Africa. ILRI (aka ILCA and ILRAD), Nairobi.

Nyahangare, E.T., Mvumi, B.M. and Mutibvu, T., 2015. Ethnoveterinary plants and practices used for ecto-parasite control in semi-arid smallholder farming areas of Zimbabwe. Journal of ethnobiology and ethnomedicine, 11(1), p.30.

Nyangiwe, N., Goni, S., Hervé-Claude, L.P., Ruddat, I., Horak, I.G., 2011. Ticks on pastures and on two breeds of cattle in the Eastern Cape province, South Africa. Onderstepoort Journal of Veterinary Research 78, 1-9.

Nyangiwe, N., Harrison, A., Horak, I.G., 2013a. Displacement of *Rhipicephalus decoloratus* by *Rhipicephalus microplus* (Acari: Ixodidae) in the Eastern Cape Province, South Africa. Experimental and Applied Acarology 61, 371-382.

Nyangiwe, N., Matthee, C., Horak, I., Matthee, S., 2013b. First record of the pantropical blue tick *Rhipicephalus microplus* in Namibia. Experimental and Applied Acarology 61, 503-507.

Ochanda, H., Young, A.S., Medley, G.F., Perry, B.D., 1998. Vector competence of 7 rhipicephalid tick stocks in transmitting 2 *Theileria parva* parasite stocks from Kenya and Zimbabwe. Parasitology 116, 539-545.

Oliver Jr, J.H., 1989. Biology and systematics of ticks (Acari: Ixodida). Annual Review of Ecology and Systematics 20, 397-430.

Olwoch, J.M., Van Jaarsveld, A.S., Scholtz, C.H., Horak, I.G., 2007. Climate change and the genus Rhipicephalus (Acari: Ixodidae) in Africa. Onderstepoort Journal of Veterinary Research 74, 45-72.

Paulauskas, A., Radzijevskaja, J., Rosef, O., Turcinaviciene, J., Ambrasiene, D., Makareviciute, D., 2006. Genetic variation of ticks (*Ixodes ricinus* L.) in the Lithuanian and Norwegian populations. Experimental and Applied Acarology 40, 259-270.

Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics 28.

Pearce, J., Ferrier, S., 2000. Evaluating the predictive performance of habitat models developed using logistic regression. Ecological modelling 133, 225-245.

Pegram, R.G., James, A.D., Bamhare, C.F., Dolan, T.T., Hove, T.F., Kanhai, G.K., Latif, A.A., 1996. Effects of immunisation against *Theileria parva* on beef cattle productivity and economics of control options. Tropical Animal Health and Production 1, 99-111.

Pegram, R.G., Tatchell, J.J., De Castro, H.G.B., Chizyuka, M.J., Creek, P.J., McCosker, M.C., Nigarura, M., Nigarura, G., 1993. Tick control: New concepts. World Animal Review 74/75, 2-11.

Pegram, R.G., Wilson, D.D., Hansen, J.W., 2000. Past and present national tick control programs. Why they succeed or fail. Annals of New York Academy of Sciences 916, 546-554.

Perez, F., 2004. Serial Cloner v. 2.6. 0. In. Software SerialBasics.

Perry, B.D., Kruska, R., Lessard, P., Norval, R.A.I., Kundert, K., 1991a. Estimating the distribution and abundance of *Rhipicephalus appendiculatus* in Africa. Preventive Veterinary Medicine 11, 261-268.

Perry, B.D., Norval, R.A.I., Kruska, R.L., Ushewokunze-Obatolu, U., Booth, T.H., 1991b. Predicting the epidemiology of tick-borne diseases of cattle in Zimbabwe using geographic information systems. In: Proceedings of the 6th ISVEE, International Symposium on Veterinary Epidemiology and Economics, pp. 214-216.

Peter, R.J., Van den Bossche, P., Penzhorn, B.L., Sharp, B., 2005. Tick, fly, and mosquito control lessons from the past, solutions for the future. Veterinary Parasitology 132, 205-215.

Peter, T.F., Perry, B.D., O'Callaghan, C.J., Medley, G.F., Mlambo, G., Barbet, A.F., Mahan, S.M., 1999. Prevalence of *Cowdria ruminantium* infection in *Amblyomma hebraeum* ticks from heartwater-endemic areas of Zimbabwe. Epidemiology and Infection 123, 309-316.

Peter, T.F., Perry, B.D., O'Callaghan, C.J., Medley, G.F., Shumba, W., Madzima, W., Burridge, M.J., Mahan, S.M., 1998a. Distributions of the vectors of heartwater, *Amblyomma hebraeum* and *Amblyomma variegatum* (Acari: Ixodidae), in Zimbabwe. Experimental and Applied Acarology 22, 725-740.

Peter, T.F., Perry, B.D., O'Callaghan, C.J., Medley, G.F., Shumba, W., Madzima, W., Burridge, M.J., Mahan, S.M., 1998b. The distribution of heartwater in the highveld of Zimbabwe, 1980-1997. Onderstepoort Journal of Veterinary Research 65, 177-187.

Petney, T., Horak, I.G., Rechav, Y., 1987. The ecology of the African vectors of heartwater, with particular reference to *Amblyomma hebraeum* and *Amblyomma variegatum*. Onderstepoort Journal of Veterinary Research 54, 381-395.

Pfäffle, M., Littwin, N., Muders, S.V., Petney, T.N., 2013. The ecology of tick-borne diseases. International Journal for Parasitology 43, 1059-1077.

Pfister, K., Armstrong, R., 2016. Systemically and cutaneously distributed ectoparasiticides: a review of the efficacy against ticks and fleas on dogs. Parasites & Vectors 9, 436.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945-959.

Pruett, J.H., 2002. Comparative inhibition kinetics for acetylcholinesterases extracted from organophosphate resistant and susceptible strains of *Boophilus microplus* (Acari: Ixodidae). Journal of economic entomology, 95(6), pp.1239-1244.

Pruett JH, Guerrero FD, Hernandez R. Isolation and identification of an esterase from a Mexican strain of *Boophilus microplus* (Acari: Ixodidae). J Econ Entomol 2002; 95(5): 1001-1007

QGIS Development Team, 2013. QGIS geographic information system. Open Source Geospatial Foundation Project http://qgis. osgeo. org.

R Development Core Team, 2013. R: A language and environment for statistical computing. In. R Foundation for Statistical Computing, Vienna, Austria.

Rajput, Z.I., Hu, S.h., Chen, W.j., Arijo, A.G., Xiao, C.w., 2006. Importance of ticks and their chemical and immunological control in livestock. Journal of Zhejiang University. Science. B 7, 912-921.

Randolph, S.E., Storey, K., 1999. Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. Journal of Medical Entomology 36, 741-748.

Randolph, S.E., 2000. Ticks and tick-borne disease systems in space and from space. Advances in parasitology 47, 217-243.

Randolph, S.E., 2010. Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129, S37-S65.

Rechav, Y., Norval, R.A.I., Oliver, J.H., 1982. Interspecific mating of *Amblyomma hebraeum* and *Amblyomma variegatum* (Acari: Ixodidae). Journal of Medical Entomology 19, 139-142.

Regassa, A., de Castro, J.J., 1993. Tick resistance to acaricides in western Ethiopia. Tropical Animal Health and Production 25, 69-74.

Rege, J.E.O., 2001. Zebu cattle of Kenya: Uses, performance, farmer preferences, measures of genetic diversity and options for improved use. ILRI (aka ILCA and ILRAD), Nairobi.

Rich, K.M., Perry, B.D., 2012. Controlling Animal Disease in Africa. In: Health and Animal Agriculture in Developing Countries. Springer, London, pp. 305-325.

Rikhotso, B.O., Stoltsz, W.H., Bryson, N.R., Sommerville, J.E., 2005. The impact of 2 dipping systems on endemic stability to bovine babesiosis and anaplasmosis in cattle in 4 communally grazed areas in Limpopo Province, South Africa. Journal of South African Veterinary Association 76, 217-223.

Robbertse, L., Baron, S., van der Merwe, N.A., Madder, M., Stoltsz, W.H., Maritz-Olivier, C., 2016. Genetic diversity, acaricide resistance status and evolutionary potential of a *Rhipicephalus microplus* population from a disease-controlled cattle farming area in South Africa. Ticks and Tick Borne Diseases 7, 595-603.

Roderick, G.K. and Navajas, M., 2003. Genes in new environments: genetics and evolution in biological control. Nature Reviews Genetics, 4(11), pp.889-899.

Rosario-Cruz R, Guerrero FD, Miller RJ, Rodriguez-Vivas RI, Domínguez-Garcia DI, Cornel AJ, et al. Roles played by esterase activity and by a sodium channel mutation involved in pyrethroid resistance in populations of *Boophilus microplus* (Acari: Ixodidae) collected from Yucatán, Mexico. J Med Entomol 2005; 42(6): 1020-1025.

Rosario-Cruz, R., Almazan, C.F., Miller RJ FAU - Dominguez-Garcia, D.I., Dominguez-Garcia DI FAU - Hernandez-Ortiz, R., Hernandez-Ortiz, R.F., de la Fuente, J., 2009. Genetic basis and impact of tick acaricide resistance. Frontiers in Biosciences 14, 2657-2665.

Roush, R.T., Daly, J.C., 1990. The role of population genetics in resistance research and management. In: Pesticide resistance in arthropods. Springer, London, pp. 97-152.

Rushton, J., Piling, D., Heffernan, C., 2002. A literature review of livestock diseases and their importance in the lives of poor people. In: Perry, B.D. (Ed.), Investing in animal health research to alleviate poverty". ILRI, Nairobi.

Rushton, S.P., Ormerod, S.J., Kerby, G., 2004. New paradigms for modelling species distributions? Journal of Applied Ecology 41, 193-200.

Samish, M., Ginsberg, H. and Glazer, I., 2004. Biological control of ticks. Parasitology, 129(S1), pp.S389-S403.

Selkoe, K.A. and Toonen, R.J., 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology letters, 9(5), pp.615-629.

Scoones, I., Mahenehene, J., Marongwe, N., Mavedzenge, B., Murimbarimba, F. and Sukume, C., 2010. Zimbabwe's land reform: myths & realities. James Currey, Suffolk.

Shaw, R.D., 1966. Culture of an organophosphorus-resistant strain of *Boophilus microplus* (Can.) and an assessment of its resistance spectrum. Bulletin of Entomological Research 56, 389-405.

Shoop, W.L., Mrozik, H. and Fisher, M.H., 1995. Structure and activity of avermectins and milbemycins in animal health. Veterinary parasitology, 59(2), pp.139-156.

Short, N.J., Floyd, R.B., Norval, R.A.I. and Sutherst, R.W., 1989a. Survival and behaviour of unfed stages of the ticks *Rhipicephalus appendiculatus*, *Boophilus decoloratus* and *B. microplus* under field conditions in Zimbabwe. Experimental & applied acarology, 6(3), pp.215-236.

Short, N.J., Floyd, R.B., Norval, R.A.I. and Sutherst, R.W., 1989b. Development rates, fecundity and survival of developmental stages of the ticks *Rhipicephalus appendiculatus*, *Boophilus decoloratus* and *B. microplus* under field conditions in Zimbabwe. Experimental and Applied Acarology, 6(2), pp.123-141.

Singh, S.K. and Girschick, H.J., 2003. Tick–host interactions and their immunological implications in tick-borne diseases. Current science, pp.1284-1298.

Smeenk, I., Kelly, P.J., Wray, K., Musuka, G., Trees, A.J., Jongejan, F., 2000. *Babesia bovis* and *B. bigemina* DNA detected in cattle and ticks from Zimbabwe by polymerase chain reaction. Journal of South African Veterinary Association 71, 21-24.

Soberanes, N.C., Santamaria, M.V., Fragoso, H.S., Garcia, Z.V., 2002. First case reported of amitraz resistance in the cattle tick *Boophilus microplus* in Mexico. Tèc. Pecu. Mèx. 40, 81–92.

Sparks, T.C. and Nauen, R., 2015. IRAC: Mode of action classification and insecticide resistance management. Pesticide Biochemistry and Physiology, 121, pp.122-128.

Speybroeck, N., Madder, M., Van Den Bossche, P., Mtambo, J., Berkvens, N., Chaka, G., Mulumba, M., Brandt, J., Tirry, L., Berkvens, D., 2002. Distribution and phenology of ixodid ticks in southern Zambia. Medical and Veterinary Entomology 16, 430-441.

Spickett, A.M., De Klerk, D., Enslin, C.B. and Scholtz, M.M., 1989. Resistance of Nguni, Bonsmara and Hereford cattle to ticks in a bushveld region of South Africa.

Spickett, A.M. and Fivaz, B.H., 1992. A survey of cattle tick control practices in the eastern Cape Province of South Africa.

Spickett, A.M., Heyne, I.H., Williams, R., 2011. Survey of the livestock ticks of the North West province, South Africa. Onderstepoort Journal of Veterinary Research 78, 305.

Stone, B.F., Haydock, K.P., 1962. A method for measuring the acaricide-susceptibility of the cattle tick *Boophilus microplus* (Can.). Bulletin of Entomological Research 53, 563-578.

Stone, N.E., Olafson, P.U., Davey, R.B., Buckmeier, G., Bodine, D., Sidak-Loftis, L.C., Giles, J.R., Duhaime, R., Miller, R.J., Mosqueda, J., Scoles, G.A., Wagner, D.M., Busch, J.D., 2014. Multiple mutations in the para-sodium channel gene are associated with pyrethroid resistance in *Rhipicephalus microplus* from the United States and Mexico. Parasites & Vectors 7, 456-468.

Suiter, D.R. and Scharf, M.E., 2009. Insecticide basics for the pest management professional. University of Georgia Cooperative Extension.

Sutherst, R.W., 1987. The dynamics of hybrid zones between tick (Acari) species. International Journal for Parasitology 17, 921-926.

Sutherst, R.W., Maywald, G.F., 1985. A computerised system for matching climates in ecology. Agriculture, Ecosystems & Environment 13, 281-299.

Swanepoel, R., A. J. Shepherd, P. A. Leman, S. P. Shepherd, G. M. McGillivray, M. J. Erasmus, L. A. Searle, and D. E. Gill. 1987. "Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa." The American journal of tropical medicine and hygiene, 36 (1): 120-132.

Tabachnick, W.J. and Black IV, W.C., 1995. Making a case for molecular population genetic studies of arthropod vectors. Parasitology today, 11(1), pp.27-30.

Tatchell, R.J., 1992. Ecology in relation to integrated tick management. International Journal of Tropical Insect Science, 13(4), pp.551-561.

Taylor, M.A., 2001. Recent developments in ectoparasiticides. The Veterinary Journal, 161(3), pp.253-268.

Tavirimirwa, B., Mwembe, R., Ngulube, B., Banana, N.Y.D., Nyamushamba, G.B., Ncube, S., Nkomboni, D., 2013. Communal cattle production in Zimbabwe: A review. Livestock Research for Rural Development 25.

Temeyer, K.B., Davey, R.B. and Chen, A.C., 2004. Identification of a third *Boophilus microplus* (Acari: Ixodidae) cDNA presumptively encoding an acetylcholinesterase. Journal of medical entomology, 41(3), pp.259-268.

Temeyer, K.B., Pruett, J.H. and Olafson, P.U., 2010. Baculovirus expression, biochemical characterization and organophosphate sensitivity of rBmAChE1, rBmAChE2, and rBmAChE3 of *Rhipicephalus (Boophilus) microplus*. Veterinary parasitology, 172(1), pp.114-121.

Thornton, P.K., 2010. Livestock production: recent trends, future prospects. Philosophical Transactions of the Royal Society B: Biological Sciences 365, 2853-2867.

Thrusfield, M.V., 2005. Veterinary Epidemiology. Blackwell Publishing, Oxford.

Tóth, G., Gáspári, Z. and Jurka, J., 2000. Microsatellites in different eukaryotic genomes: survey and analysis. Genome research, 10(7), pp.967-981.

Tønnesen, M.H., Penzhorn, B.L., Bryson, N.R., Stoltsz, W.H., Masibigiri, T., 2004. Displacement of *Boophilus decoloratus* by *Boophilus microplus* in the Soutpansberg region, Limpopo Province, South Africa. Experimental and Applied Acarology 32, 199-208.

Tuppurainen, E.S.M., Stoltsz, W.H., Troskie, M., Wallace, D.B., Oura, C.A.L., Mellor, P.S., Coetzer, J.A.W., Venter, E.H., 2011. A Potential Role for Ixodid (Hard) Tick Vectors in the Transmission of Lumpy Skin Disease Virus in Cattle. Transboundary and Emerging Diseases 58, 93-104.

Uilenberg, G., 1976. Epizootiology of tick-borne diseases. World Animal Review. 17,8-15.

Uilenberg, G., 1999. Immunization against diseases caused by Theileria parva. Tropical Medicine and International Health 4, A12-A20.

Uilenberg, G., 2006. Babesia historical overview. Veterinary Parasitology 138, 3-10.

Van Houtte, N., Van Oosten, A.R., Jordaens, K., Matthysen, E., Backeljau, T., Heylen, D.J.A., 2013. Isolation and characterization of ten polymorphic microsatellite loci in Ixodes arboricola, and cross-amplification in three other Ixodes species. Experimental and Applied Acarology 61, 327-336.

Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W. and Tirry, L., 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. Insect biochemistry and molecular biology, 40(8), pp.563-572.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P., Shipley, P., 2004. MICRO - CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular ecology notes 4, 535-538.

Vudriko, P., Okwee-Acai, J., Tayebwa, D.S., Byaruhanga, J., Kakooza, S., Wampande, E., Omara, R., Muhindo, J.B., Tweyongyere, R., Owiny, D.O. and Hatta, T., 2016. Emergence of multi-acaricide resistant *Rhipicephalus* ticks and its implication on chemical tick control in Uganda. Parasites & vectors, 9(1), p.4.

Vudriko, P., Umemiya-Shirafuji, R., Okwee-Acai, J., Tayebwa, D.S., Byaruhanga, J., Jirapattharasate, C., Liu, M., Moumouni, P.F.A., Fujisaki, K., Xuan, X. and Suzuki, H., 2017. Genetic mutations in sodium channel domain II and carboxylesterase genes associated with phenotypic resistance against synthetic pyrethroids by *Rhipicephalus (Boophilus) decoloratus* ticks in Uganda. *Pesticide Biochemistry and Physiology*. https://doi.org/10.1016/j.pestbp.2017.07.009

Walker, A.R., Bouattour, A., Camicas, L., Estrada-Peña, A., Horak, I.G., Latif, A.A., Pegram, P.G., Preston, P.M., 2007. Ticks of Domestic Animals in Africa: a Guide to Identification of Species. Bioscience Reports, Edinburgh.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Pena, A., Horak, I.G., Latif, A.A., Pegram, R.G., Preston, P.M., 2003. Ticks of domestic animals in Africa: a guide to identification of species. Bioscience reports Edinburgh.

Walker, J.B., 1991. A review of the ixodid ticks (Acari, Ixodidae) occurring in southern Africa. Onderstepoort Journal of Veterinary Research 58, 81-105.

Walker, J.B., Keirans, J.E., Horak, I.G., 2000. The genus *Rhipicephalus* (Acari, Ixodidae): a guide to the brown ticks of the world. Cambridge University Press.

Wall, R., Shearer, D., 2001. Veterinary ectoparasites: Biology, Pathology and Control. Pathology and Control, second ed. Blackwell Science, London.

Ware, G.W., 2000. The pesticide book 5th edn. Francis Thomson Publications, Fresno, California.

Weir, B.S. and Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. evolution, 38(6), pp.1358-1370.

Weising, K., Nybom, H., Pfenninger, M., Wolff, K., Kahl, G.Ã., 2005. DNA fingerprinting in plants: principles, methods, and applications. CRC press, Taylor and Francis Group, London.

Whitehouse, C.A., 2004. Crimean–Congo hemorrhagic fever. Antiviral research, 64(3), pp.145-160.

Willadsen, P., Bird, P., Cobon, G.S. and Hungerford, J., 1995. Commercialisation of a recombinant vaccine against *Boophilus microplus*. Parasitology, 110(S1), pp.S43-S50.

Willadsen, P., 2006. Tick control: thoughts on a research agenda. Veterinary Parasitology 138, 161-168.

Wilson, A., 1996. Appropriate Strategies for the Control or Eradication of Ticks and Tick Borne Diseases. Annals of the New York Academy of Sciences 791, 54-63.

Wyk, R.D., Baron, S., Maritz-Olivier, C., 2016. An integrative approach to understanding pyrethroid resistance in *Rhipicephalus microplus* and *R. decoloratus* ticks. Ticks and Tick Borne Diseases 7, 586-594.

Young, A.S., Groocock, C.M., Kariuki, D.P., 1988. Integrated control of ticks and tick-borne diseases of cattle in Africa. Parasitology 96, 403-432.

Zeman, P., Lynen, G., 2010. Conditions for stable parapatric coexistence between *Boophilus decoloratus* and *B. microplus* ticks: a simulation study using the competitive Lotka-Volterra model. Experimental and Applied Acarology 52, 409-426.

Curriculum Vitae

Personal Information

Name:	Marvelous Sungirai
Date of birth:	29 January 1984
Place of birth:	Bulawayo, Zimbabwe
Nationality:	Zimbabwean
Email:	sungiraim@staff.msu.ac.zw / sungiraim@gmail.com

Education

2013-2017 PhD Candidate, Ghent University Faculty of Applied Biological Sciences and Bioscience engineering.

Thesis: Ixodid ticks parasitising cattle in Zimbabwe: Ecology and management

Promoters: Professor Maxime Madder, Professor Doreen Zandile Moyo and Professor Patrick De Clercq

2012-2013- Post-graduate Diploma in Tertiary Education. Midlands State University, Zimbabwe.

2011-2012-MSc Tropical Animal Health, Institute of Tropical Medicine, Belgium.

2003-2006- BSc(Hon) Livestock and Wildlife Management, Midlands State University, Gweru, Zimbabwe.

Other credit courses: Tick identification (University of Pretoria), Multivariable Analysis (Institute of Tropical Medicine), Applied Molecular Epidemiology of Infectious Diseases (Institute of Tropical Medicine), Basic Epidemiology (Institute of Tropical Medicine), Advanced Epidemiology (Institute of Tropical Medicine), Applied Epidemiology (Institute of Tropical Medicine).

Professional career

2009-2012-Assistant Lecturer, Department of Livestock and Wildlife Management, Midlands State University, Gweru, Zimbabwe.

2012-present- Lecturer, Department of Livestock and Wildlife Management, , Midlands State University, Gweru, Zimbabwe.

Publications

1. **Marvelous Sungirai**, Samantha Baron, Doreen Zandile Moyo, Patrick De Clercq, Christine Maritz-Olivier, Maxime Madder. (2017) Genotyping acaricide resistance profiles of *Rhipicephalus microplus* tick populations from communal land areas of Zimbabwe, Ticks and Tick-borne Diseases., ISSN 1877-959X, https://doi.org/10.1016/j.ttbdis.2017.10.01
2. **Sungirai, M**., Abatih, E. N., Moyo, D. Z., De Clercq, P. and Madder, M. (2016), Shifts in the distribution of ixodid ticks parasitizing cattle in Zimbabwe. Med Vet Entomol. doi:10.1111/mve.12215
3. **Sungirai, M**., Moyo, D. Z., De Clercq, P., & Madder, M. (2016). Communal farmers' perceptions of tick-borne diseases affecting cattle and investigation of tick control methods practiced in Zimbabwe. Ticks and Tick-borne diseases, 7(1), 1-9.
4. **Sungirai, M**., Masaka, L., Nyamukanza, C., Chimwaza, E.P. (2015). Effect of grouping weaned piglets by weight classes: Evidence from Zimbabwe. Midlands State University Journal of Science, Agriculture and Technology.
5. Masaka, L., Mhaka, V., **Sungirai, M**., & Nyamukanza, C. (2015). Growth performance of Brangus steers fed graded levels of sun-dried broiler litter as a substitute for cottonseed cake. *Tropical animal health and production*, 1-5. DOI10.1007/s11250-015-0827-2.
6. **Sungirai, M**., Madder, M., Moyo, D. Z., De Clercq, P., & Abatih, E. N. (2015). An update on the ecological distribution of the Ixodidae ticks in Zimbabwe. *Experimental and Applied Acarology*, 1-12. DOI. 10.1007/s10493-015-9892-5.
7. Timothy Gotora, Lawrence Masaka, and **Marvelous Sungirai**, "Effect of Cow Urine on the Growth Characteristics of *Fusarium lateritium*, an Important Coffee Fungus in Zimbabwe," *International Journal of Agronomy*, vol. 2014, Article ID 986068, 4 pages, 2014. doi:10.1155/2014/986068
8. **Marvelous Sungirai**, Lawrence Masaka, and Tonderai Maxwell Benhura, "Validity of Weight Estimation Models in Pigs Reared under Different Management Conditions," *Veterinary Medicine International*, vol. 2014, Article ID 530469, 5 pages, 2014. doi:10.1155/2014/530469

9. Lawrence Masaka, **Marvelous Sungirai,** Casper Nyamukanza & Chido Bhondai. 2014. Sow removal in a commercial pig herd in Zimbabwe. *Tropical Animal Health and Production.* DOI:10.1007/s11250-014-0554-0.
10. **Sungirai M**., Masaka L. & Mbiba C. 2014. The prevalence of *Taenia saginata* cysticercosis in the Matabeleland Provinces of Zimbabwe. *Tropical Animal Health and Production*. DOI:10.1007/s11250-014-0538-0. Impact Factor 1.09
11. **Marvelous Sungirai**, Moses Mucheni, Lawrence Masaka 2013. Efficacy of different concentrations of *Aloe chabaudii* leaf gel as a substitute of a sulphanomide for the control Avian Coccidiosis**.***Open Journal of Applied Sciences.*7:3 pp 430-435. http://dx.doi.org/10.4236/ojapps.2013.37053
12. De Clercq E. M., Vanwambeke S. O., **Sungirai M**., Adehan S., Lokossou R. & Madder M. 2012. Geographic distribution of the invasive cattle tick Rhipicephalus *microplus*, a country-wide survey in Benin. *Experimental and Applied Acarology*. DOI 10.1007/s10493-012-9587-0.
13. **Sungirai, M.** and Ngwenya, M., 2016. An Investigation into the Efficiency of Utilization of Artificial Game Water Supplies by Wildlife Species in the North Eastern Kalahari Region of Hwange National Park in Zimbabwe. Applied Ecology and Environmental Sciences, 4(1), pp.7-14.